Handbook of Blood Banking & Transfusion Medicine

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Published by
Jitendar P Vij
Jaypee Brothers Medical Publishers (P) Ltd
EMCA House, 23/23B Ansari Road, Daryaganj
New Delhi 110 002, India
Phones: +91-11-23272143, +91-11-23272703, +91-11-23282021, +91-11-23245672
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Handbook of Blood Banking and Transfusion Medicine
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First Edition: 2006
ISBN 81-8061-718-1
Typeset at JPBMP typesetting unit
Printed at Replika Press Pvt. Ltd.
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This book of 26 chapters is designed to share current topics in blood banking, transfusion medicine and stem cell transplantation. This book will serve as a guide to individuals in the blood banking profession and, equally so, will be helpful to those physicians who transfuse blood and those entering the field of stem cell transplantation. The authors are outstanding experts in their fields who have chosen to contribute their efforts, so current blood banking topics are available and that the price of the book is more affordable than those published in the USA and Europe. I thank these authors for their generosity and expertise.

The authors of four chapters are experts from India, with another by authors from Geneva but the remaining 21 chapters were from Minneapolis, Minnesota, USA, written mainly by current faculty members or past transfusion medicine fellow trainees from the University of Minnesota Medical School in Minneapolis, Minnesota. This book brings to the reader the experience of the physicians from an academic tertiary care center where approximately 200 stem cell transplants have taken place annually for the past 15 years, where over 50,000 platelets concentrates are transfused each year, where organ transplantation innovation is commonplace and where teaching and research are a proud tradition. We have included the blood component use guidelines that are in use at the Fairview-University Medical Center at the University of Minnesota Medical School. These are guidelines that have been agreed upon by the medical staff, approved by the hospital transfusion committee and are revised at intervals. They represent useful audit criteria to initiate peer review of transfusion appropriateness.

This book contains 20 chapters of up-to-date, useful information about general blood banking topics, blood components, clinical use and complications of blood component transfusion followed by six chapters about stem cell transplantation. These latter chapters are devoted to transplantation of hematopoietic stem cells derived from marrow, peripheral blood and the placenta. Topics include the use of bone marrow transplants to treat disease, transplants from unrelated donors, stem cell processing and storage, cord blood banking and stem cell infusions.

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Acknowledgements

The editors acknowledge thanks to all the contributors, who provided articles and Power Point presentations for these projects. We also thank the Minneapolis-University Rotary Club, The Rotary Club of Bangalore and the Rotary/TTK Blood Bank for their financial support of this project. We appreciate the excellent job done in publishing the *Handbook of Blood Banking and Transfusion Medicine* and the CD ROM as a teaching guide for the Blood Bank and Transfusion Medicine Staff, by M/s Jaypee Brothers Medical Publishers (P) Ltd, EMCA House 23/23B, Ansari Road, New Delhi, 110 002, India. We also thank the staff of TRIGENT, a software development company in Bangalore, India, for their help in developing our website.
Contents

BLOOD BANKING

1. Transfusion Medicine of Today and the Future
   Jeffrey J McCullough
   1

2. WHO Initiatives on Safe Blood Programs in the Developing Countries
   Neelam Dhingra, Noryati Abu Amin, Jan Fordham
   5

3. Organization and Operation of a Regional Blood Transfusion Center in India
   Latha Jagannathan
   11

4. Blood Donor Suitability and Donation Complications
   Bruce Newman
   27

5. Leukoreduction of Blood Components
   John P Miller
   36

6. Platelets in Health and Disease
   Sunny Joseph Varghese
   64

7. Recent Advances in Platelet Preservation and Testing
   Daniel Ericson, Gundu HR Rao
   87

8. The HLA System and Transfusion Medicine
   S Yoon Choo
   98

9. Transfusion-associated Graft-vs-Host Disease
   Jed B Gorlin
   110

CLINICAL USE OF BLOOD COMPONENTS

10. Blood Component Administration and Initial Management of Transfusion Reactions
    Ted Eastlund, Kathrine Frey
    122

    Ted Eastlund, Kathrine Frey, David Mair
    133

12. Transfusions in Critically Ill Patients
    Abram H Burgher, Jeffrey G Chipman
    146

13. Blood Transfusion in the Operating Room
    David Beebe, Kumar Belani
    159

14. Massive Transfusions
    John Crosson
    165

15. Transfusion Therapy for Hemoglobinopathies
    Anil V Pathare
    173
HEMATOPOIETIC STEM CELL TRANSPLANTATION

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.</td>
<td>Hematopoietic Stem Cell Transplantation for Malignant Diseases</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td><em>Mukta Arora, Chatchada Karanes</em></td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>Unrelated Donor Stem Cell Transplantation: The Role of the National Marrow Donor Program</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td><em>Chatchada Karanes, Tim Walker</em></td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Umbilical Cord Blood Banking</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td><em>Sabeen Askari, David McKenna, John P Miller</em></td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>Hematopoietic Stem Cell Processing</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td><em>David H McKenna Jr, Mary E Clay</em></td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td>Infusion of Hematopoietic Stem Cells</td>
<td>284</td>
</tr>
<tr>
<td></td>
<td><em>Annette Sauer-Heilborn</em></td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>Bone Marrow Transplantation: Procedures and Practices in a Developing Country</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td><em>Mammen Chandy, David Dennison</em></td>
<td></td>
</tr>
</tbody>
</table>

Index 303
Introduction

In the summer of 1998 Rotarian Mrs Deborah Watts and I visited South Africa with the help of a discovery grant from the Rotary International, to learn about the Transfusion Medicine Practices and the prevalence of blood-borne diseases. We found to our pleasant surprise that the transfusion medicine services as practiced in South Africa is one of the best in the world. During the same time, I also visited several blood banks and transfusion medicine centers in India. According to rough estimates as well as published information, anywhere from 3 to 6 percent of the blood collected is contaminated with pathogens. In one of the publications, it was demonstrated that using good sterile way of drawing blood, reduced \textit{Staphylococcus epidermis} infection, by fifty percent. Good donor screening, sterile drawing of blood and use of good manufacturing practices in processing of collected blood, have eliminated 90 percent of the bacterial contamination in the world blood supply. In view of these observations, we at the Minneapolis-University Rotary Club developed a matching grant proposal to the Rotary International to procure funds, to help facilitate the establishment of an International Training Center at the Rotary/TTK Blood Bank, Bangalore. The funds obtained was used for improving the training center at the Rotary/TTK Blood Bank as well as for publishing a book on \textit{Handbook of Blood Banking and Transfusion Medicine} (Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, India, 2005, ISBN # Editors: Prof Gundu HR Rao, Prof Ted Eastlun and Dr Latha Jagannathan). Part of these funds was used for purchasing 250 copies of the book for free distribution. The book is dedicated to the Rotary International. The book also includes a CD ROM for teaching purposes. To keep the blood bank staff abreast of the knowledge and developments in the area of blood banking and transfusion medicine, we decided to develop a web-based distance learning program (www.blood-biosafety.com). We welcome you readers, for constructive comments, so that we not only keep it updated, but keep it relevant to the needs of developing nations.

Rotary Club has played a significant role in supporting the establishment of blood banks in the developing countries. It has helped many blood banks in the developing world with funds towards the purchase of needed equipment. We wanted to give due credit to the Rotary International for their continued support of the safe blood project. However, due to privacy policy and lack of organized data at the headquarters of Rotary International, I was not able to get information about all the blood banks that have received financial support from Rotary Clubs. However, from whatever data that the fund development branch of the Rotary International was willing to share, we could safely say that blood banks in India, Nigeria, Bangladesh, Thailand, Hong Kong, Indonesia and Nepal have received significant funds from Rotary Clubs. I have included additional data that I was able to obtain from the google search engine in the section on links. If data are made available from the Rotary International, we would like to include the names of all the blood banks that have received funds from Rotary International and establish a network of Rotary Blood Banks. We would like to see that the Rotary International play a much bigger role in the development of Safe Blood Initiative, similar to their role in the eradication of polio from the world (PolioPlus).

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Transfusion medicine can involve sophisticated therapy and blood banks can be complex operations. Exciting developments are occurring in transfusion medicine that portends an exciting and different future. However, blood banks also face many difficult issues and many parts of the world have an inadequate or unsafe blood supply.

**TRANSFUSION MEDICINE AND BLOOD BANKING RESEARCH**

Many of the exciting developments that will advance the practice of transfusion medicine are due to developments in the underlying basic science, which signifies the increasingly interdisciplinary nature of transfusion medicine research. This research involves a broad spectrum that includes basic science, biomedical engineering, applied or translational work, methods, development, clinical trials, epidemiology, public health, and social sciences. To add further complexity, transfusion medicine research in basic sciences is not limited to a single discipline but is carried out by a wide variety of scientists.

There are many examples of how work in the basic sciences has had a major impact in transfusion medicine. For example, understanding of platelet structure function, physiology, and the role of membrane phospholipids have led to improved platelet preservation. Studies of red cell structure and hemoglobin function have led to improved red cell preservation, an appreciation of the control of oxygen release by stored red cells, and have been the basis for development of red cell substitutes, although this work has been difficult and progress is slow. Molecular genetics and sophisticated biochemical techniques have made it possible to identify the composition and structure of many blood group antigens and even the function of some of these molecules. Improved understanding of immunology has added to the ability to manage and prevent alloimmunization, although interestingly more than 40 years after the clinical availability of Rh (Rhesus) immune globulin for the prevention of hemolytic disease of the newborn, its basic mechanism of action is not known. Studies of the immunologic effects of transfusion have not yet defined completely the molecular and cellular basis of the response but will ultimately answer present unresolved issues regarding the role of transfusion in postoperative infection, cancer recurrence, and the success of transplanted organs. Other developments in basic immunology are being translated into clinical practice through studies of adoptive immunotherapy. Biochemistry is the basis of exciting work on inactivation of viruses and bacteria in traditional cellular blood components. New strategies for donor testing using molecular diagnostic methods to identify donors in the window phase of infection are based on biochemical, immunologic, and molecular biologic techniques developed in the basic science laboratory to detect DNA (deoxyribonucleic acid) or RNA (ribonucleic acid). The basic scientific studies identifying and developing hematopoietic growth factors such as erythropoietin, granulocyte colony-stimulating factor, and platelet growth factors have had a huge impact on clinical transfusion medicine. Cellular engineering using these and other hematopoietic growth factors and cytokines, along with an increased understanding of the
mechanisms of cell division and cell culture techniques, are enabling transfusion medicine to provide novel blood components. Studies of the function of hematopoietic stem cells and lymphocyte function have led to the use of umbilical cord blood for hematopoietic reconstitution and the use of stem cells as a blood component. Biochemical and cell membrane studies form the basis of efforts to enzymatically cleave ABO (group O, A, B and AB) antigens from red cells or to cover those antigens—two strategies for creating a universal red cell type.

Examples of other types of transfusion medicine research sometimes carried out in collaboration with other disciplines include: biomedical engineering studies of blood filtration systems, apheresis technology and instrumentation, infusion pumps, blood testing instruments and blood warmers; translational research studies implementing pathogen inactivation technology; clinical laboratory research involving improved red cell, platelet and neutrophil serologic techniques; clinical trials of new blood components; blood collection techniques such as red cell apheresis; clinical studies of transfusion complications and reactions, and donor adverse reactions; epidemiologic studies of transfusion transmitted diseases, donor demographics and emerging potential transfusion transmitted diseases; and social science studies of donor motivation, volunteerism and the public’s tolerance for risk.

Although its start was with Landsteiner’s discovery of the ABO blood group system, now in the 21st century, transfusion medicine has progressed from its beginnings. Watershed discoveries in transfusion medicine include: the understanding of hemolytic disease of the newborn by Levine and Stetson, the development of plastic containers by Walter that made blood component therapy possible, the development of plasma fractionation by Cohn that led to the availability of plasma derivatives such as albumin, immune globulins and coagulation factor concentrates, the discovery of hundreds of red cell antigens, the development of Rh immune globulin that made hemolytic disease of the newborn a preventable disease, the development of blood cell separation equipment that made apheresis a routine method of preparing some blood components, understanding transfusion transmitted diseases and the development of strategies to minimize disease transmission, and the use of hematopoietic stem cells as a blood component.

Today’s transfusion medicine is sophisticated and complex hematotherapy involving an understanding of a variety of scientific disciplines in addition to the medical management of patients.

**TRANSFUSION MEDICINE/BLOOD BANK PHYSICIANS**

Physicians with expertise and leadership will be essential for the continued improvement of the world’s blood supply and transfusion therapy. Many activities require transfusion medicine expertise, such as medical consultation regarding use of traditional blood components; therapeutic apheresis; immunohematology clinical consultation; education of hospital medical staff; administrative roles in the hospital; development and implementation of novel blood collection techniques; donor medical issues with increased collection complexity; consultation on component therapy for novel situations; technology implementation; and cellular engineering for production of new blood component. For instance, immunohematology clinical consultation can involve autoimmune hemolytic anemia, urgent transfusion for patients with red cell antibodies, rare antibodies, platelet and neutrophil serology. Education of hospital medical staff including anesthesiologists, surgeons and others regarding appropriate use of blood will be necessary. There is an important administrative role in hospitals involving the transfusion or other Committees, other medical staff activities and providing advice to hospital administrators. The development and implementation of novel blood collection techniques such as two-unit red cell collection or use of devices allowing selection of a unique component combination for each donation will need medical involvement. These advances in blood collection may also involve the use of hematopoietic growth factors and will require the establishment of policies for donor medical evaluation and selection and the prevention and management of reactions and complications.

Component therapy for novel situations such as extracorporeal membrane oxygenators (ECMOs), more complex cardiovascular surgery such as placement of left ventricular assist devices, the continued expansion of hematopoietic cell and organ transplants and other medical advances will require transfusion medicine consultation. Implementation of
these new technologies will require leadership from transfusion medicine/blood banking experts. Cellular engineering for production of new blood components is an area in great need of medical/scientific leadership for methods, development, problem-solving and laboratory management, and interaction with clinical physicians.

TRANSFUSION MEDICINE AND BLOOD BANKING WORLDWIDE

Unfortunately, the kind of transfusion therapy described above is available only in some parts of the world. The blood supply is inadequate in most developing and least developed countries. About 80 percent of the world’s population has access to only about 20 percent of the world’s supply of safe screened blood. In the early 1980s, worldwide blood collection was about 76 million units. Donations ranged from 15.2 per 1,000 population in industrial market countries, 9.5 in middle income countries, and 1.1 in low income countries. In the 1990s, Westphal reported an updated estimate from the World Health Organization (WHO) of worldwide blood production to 90 million units per year. He also estimated blood collection per 1,000 population as 50 in developed countries, 5–15 in developing countries, and 1–5 in least developed countries.

Many countries depend to a large extent on “family donations”—that is, blood donated by family or friends of the patient, but these friends are often paid by the patient’s family to provide needed blood. Many reports confirm that paid donors and these “replacement” donors have a higher prevalence of transfusion-transmitted infection. Thus, replacement donations in fact often constitute a hidden form of paid donation. Creating a stable base of volunteer donors is the biggest challenge being faced in developing countries. A focus on developing a volunteer donor base is one of the most fundamental issues in developing a safe and effective blood supply. However, a sufficient number of well-trained donor recruiters to create an adequate blood supply is usually neglected or not available. For instance, only 16 developing and least developed countries provide training for donor recruiters and there are few established posts for such individuals.

Laboratory testing for transmissible diseases is well-established, complex, and sophisticated. However, the enormous gap in the level of infections and available resources makes the screening strategies developed in Western countries both inadequate and unaffordable in developing countries. Up to 13 million units per year of the global blood supply apparently were not screened for all relevant transfusion-transmissible infections during the 1990s. For instance, the WHO estimates that screening for human immunodeficiency virus (HIV) is carried out in 100 percent of developed, 66 percent of developing countries, and 46 percent of least developed countries, and hepatitis B in 100 percent of developed, 72 percent of developing, and 35 percent of least developed countries. According to other WHO estimates, 43 percent of the blood collected in the developing world is not adequately screened. This means that about 80 percent of the world’s population has access to only 20 percent of the global supply of safe screened blood. It is, therefore, critical for developing countries to design their own blood safety strategies adapted to their epidemiological, social, and economic circumstances instead of trying to reach the inappropriate standards developed by and adapted to developed countries. For instance, rapid tests may be used in resource-poor settings and even done predonation so that infectious individuals do not go through the donation process.

The government’s commitment is necessary to develop an effective blood program. Ultimately, the government bears the responsibility for the blood transfusion system regardless of the organization which is responsible for the implementation of the program. The resources important to blood centers are not only traditional quantifiable, such as financial and infrastructure, but others that are intangible or unquantifiable such as expertise and the local, social, and cultural settings; community understanding; and acceptance of blood donation. To some extent, these resources may be lacking in all countries, although in a different format. Another approach to matching blood supply and demand is “to ensure the rationale use of blood and blood products”. Most developing and least developed countries do not have transfusion guidelines or recommendations; and often, the trans-
fusin medicine expertise necessary to educate physi-
cians regarding the appropriate use of blood products
is not available. Thus, transfusion medicine education
programs for physicians who use blood for their
patients can be helpful.

CONCLUSION

As we begin the new century, transfusion medicine
seems to be on the threshold of additional major
changes with the implementation of many of the
research developments described above. These
changes will add to the complexity of transfusion
medicine and should continue to provide exciting
opportunities for transfusion medicine physicians,
scientists, and technologists in the future. In the
developing and least developed countries of the
world, transfusion medicine and blood banking is far
different than that which occurs in developed
countries. While complex activities will be taking place
in some parts of the world, transfusion medicine
physicians in other parts of the world will be working
with governments, volunteers, and local organizations
to increase blood donations, increase the testing of
donated blood, and make transfusion therapy
available, safely, to more patients. Transfusion medi-
cine and blood banking needs leaders worldwide.

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ISSUES AND CHALLENGES IN BLOOD SAFETY IN DEVELOPING COUNTRIES

Blood transfusion is an essential element of a health care system. Millions of lives are saved each year through blood transfusions. Inadequacies in blood safety and supply contribute significantly to the burden of disease and loss of life. Safety of blood transfusion is of extreme importance in order to avoid any serious morbidity or mortality in the patient and to contribute towards saving the life of the patient. The World Health Organization (WHO) Global Database on Blood Safety (GDBS) 2000-2001, based on data from 178 countries, shows that about 81 million units of whole blood and 20 million liters of plasma are collected annually. Only 39 percent of the global blood supply is collected in the developing world where 82 percent of the world’s population lives, indicating serious lack of access to blood to meet the requirements of the health care systems in developing countries.1 The main reason for this lack of access to blood is extremely fragmented and uncoordinated Blood Transfusion Services (BTSs), either due to absence of national policy, plan or due to lack of adequate resources, both financial and human. From the GDBS data, only 24 percent of countries report having all major aspects of a well-organized blood transfusion service.

In addition, BTSs in many developing countries rely predominantly on family, replacement or paid donors. There is lack of organized community-based blood donor programs and blood is collected from unsafe donors. Developing countries contribute only 25 percent of the total donations from voluntary non-remunerated blood donors collected globally. Adequate testing of donated blood still remains an issue of major concerns. Of the 81 million units collected, up to 6 million tests were not done for human immunodeficiency virus (HIV), hepatitis B and C, syphilis, mainly in developing countries and 25 percent of countries report that blood has been issued without testing due to lack of reagents or test kits. Of the 40 million donations in developed countries, all were tested for infectious diseases; whereas, in the developing countries, only 43 percent of the 30 million donations were tested.2 The GDBS data also indicate that the use of whole blood is 24 times higher in developing countries than in the developed world, resulting in inadequate provision of life-saving support for patients requiring specialized treatment with blood component therapy in the developing world. This further widens the gap between supply and demand and contributes to shortages of blood and blood products for patients who really need them.

The lack of quality systems in BTSs, among other things, leads to poor donor selection and laboratory
procedures. This also leads to lack of continuous supply of reagents and test kits, lack of adequate equipment management, absence of adequate documentation systems, lack of standards and systems for monitoring and evaluation. Thus ultimately leads to poor quality and unsafe blood.

The GDBS data allow several elements of a quality system to be analyzed. At least, the following elements should be in place: a national quality system, full-time dedicated staff, written standard operating procedures, quality assurance program for blood screening and participation in an external quality assessment scheme. The GDBS data show that globally only 16 percent of countries have all of these elements fully in place with more than half of the percentage being made up of developed countries.

WHO BLOOD SAFETY PROGRAM

The WHO has identified blood safety as a priority; and in the year 2000, the World Health Day 2000 was dedicated to the theme of blood safety with the slogan “Safe blood starts with me. Blood saves lives”. Since then, there have been several initiatives to support the development of nationally-coordinated BTSs based on voluntary non-remunerated blood donors. The WHO recommends an integrated strategy for blood safety, highlighting the important role of commitment and support by national governments:

- **Organization and management**: The establishment of a nationally-coordinated BTS that can provide adequate and timely supplies of safe blood for all patients in need.
- **Low-risk blood donors**: The collection of blood only from voluntary non-remunerated blood donors from low-risk populations and the use of stringent donor selection procedures and strategies for donor retention.
- **Testing of all donated blood**: Testing for transfusion-transmissible infections, including HIV, hepatitis B and C viruses, syphilis (and Chagas’ disease and other disease markers, as appropriate); blood grouping and compatibility testing.
- **Appropriate clinical use of blood**: The prescription of blood components only when required, the use of intravenous replacement fluids (crystalloids and colloids) and other simple alternatives to blood transfusion, wherever possible, to reduce unnecessary transfusions and procedures to ensure the safe administration of the right blood to the right patient.
- **Quality systems**: The above strategies can be effective only if quality systems covering all aspects of blood transfusion are in place. The quality system must be comprehensive, from the recruitment and selection of blood donors to the transfusion of blood and blood products to patients, and should reflect the needs of the clinicians and patients served.

Advocacy for Nationally-Coordinated Blood Transfusion Programs

The government’s commitment and support for a well-organized, nationally-coordinated service is the first step in ensuring sustainability and is a prerequisite for ensuring safe blood and blood components. The WHO plays a high-level advocacy role and provides advice, technical guidance and support to countries for establishment of sustainable national blood program with sufficient financial resources and well-qualified and trained staff in blood transfusion.

The WHO is developing guidelines on Establishment of National Blood Program and Legislative Framework for the National Blood Program, to support countries in establishing national blood programs.

Costing Blood Transfusion Services

Many health authorities recognize the need for a sustainable national blood program to meet the needs for a safe and adequate blood supply. However, very few BTSs are able to collect or provide accurate and realistic information relating to capital and recurrent costs; and therefore, an adequate budget cannot be provided (whether through budgetary allocation, a cost recovery system or a combination of the two). Moreover, there is often an incorrect perception that, since blood is donated voluntarily, costs are minimal. Without cost collection and cost analysis, BTSs cannot provide governments and funding agencies with the information required to develop and maintain sustainable national blood programs.
Only with clear and concise costing tools can cost analysis and cost management be put into practice. The WHO has produced a workbook, *Costing Blood Transfusion Services* with spreadsheets, in hard copy and electronic format, in order to assist Member-States in developing costing procedures to ensure a sustainable blood program. The costing tools provided are simple, practical and comprehensive.

**Global Collaboration for Blood Safety**

The Global Collaboration for Blood Safety (GCBS) is an initiative aimed at creating improved collaboration between organizations and institutions involved in the area of blood safety.

The GCBS was launched following the Paris AIDS (Acquired Immunodeficiency Syndrome) Summit in 1994. The resolution called for improved collaboration in the area of blood safety and identified the need for a mechanism for communication between national and international organizations involved in the safety of blood and blood products; manufacturers of plasma, plasma derivatives and blood devices; users and prescribers of blood; blood donor organizations, source plasma donors and recipients of blood and blood products. The GCBS provides a forum for communication and agreement on joint and complementary action. The goal of the GCBS is to promote and strengthen international collaboration on the safety of blood products and transfusion practices. Consensus during the two plenary meetings held in 2000 and 2001 resulted in the formation of three working groups. These groups are currently developing work products including an *Aide-Mémoire: Good Policy Practice* to support decision-making in blood transfusion practice, *Provision of Plasma-Derived Medicinal Products and Minimum Requirement Standards for BTSs*.

**WHO Quality Management Program**

As part of the renewed drive for a safe and adequate global blood supply, the WHO launched a new program—the Quality Management Program (QMP) — in 2000 which has been developed as a long-term activity.

The QMP is designed to develop regional and national capacity in quality management and to promote the establishment of effective quality systems in BTSs in all WHO Member-States. The principal objective of this program is to promote the concept of quality systems and to assist BTSs in establishing and implementing quality systems including quality assurance, documentation systems, good manufacturing practices, quality control and assessment procedures. Activities carried out at global, regional and national levels include:

- Establishment of regional quality training centers
- Training of trainers
- Development of advocacy and training materials
- Training of quality officers in well-structured Quality Management Training courses
- Establishment and strengthening of External Quality Assessment Schemes (EQAS)
- Provision of post-training support and follow-up.

Key achievements in this program include the organization of 26 quality management training courses in three years, training of 79 global and regional facilitators, training of more than 470 BTS staff from 121 countries as quality officers, introduction of the program to 240 directors of blood transfusion services and increased participation of centers, now 257, in WHO-external quality assessment schemes.

As a long-term activity, the QMP’s focus for the future will be to support countries that are committed to achieving a safe and adequate blood supply for all patients requiring blood transfusions by assisting Member-States in establishing quality systems in BTSs and in monitoring, evaluating and re-planning.

**External Quality Assessment Schemes (EQAS)**

Quality management in blood transfusion laboratory practice has attained a greater significance in view of the risks associated with unsafe blood transfusions. Regional EQAS centers have been established with the objective of monitoring trends, identifying problems and implementing corrective action. Ensuring good laboratory practice based on quality assurance and quality control measures, with standardized procedures and high quality reagents, will stimulate information exchange and networking at all levels thus improving performance and accuracy.

Activities in this area include organizing regional EQAS to improve the performance of the laboratories that test donors for infectious diseases and blood groups, as well as conducting regional training workshops, identifying areas for further training and providing support wherever needs are identified.
The WHO Guidelines on External Quality Assessment Schemes (EQAS) in Blood Group Serology and Testing for Transfusion-Transmissible Infections have been developed to support countries in establishing these programs.

**Distance Learning Material: Safe Blood and Blood Products**

The WHO has developed a series of interactive distance learning materials: *Safe Blood and Blood Products* which are designed to provide an alternative means of rapidly improving the knowledge and technical skills of staff in BTSs in developing countries. Although designed for use in distance learning programs about blood safety, they can also be used for independent study or as resource materials in conventional training courses and in-service training programs. They enable BTSs to update the knowledge of staff in a practical and cost-effective way and to make effective use of limited training resources.

The materials have been produced for staff with responsibility for donor recruitment and retention and for the collection, testing and issue of blood and blood products. They comprise the following modules:

- **Introductory Module: Guidelines and Principles for Safe Blood Transfusion Practice**
- **Module 1: Safe Blood Donation**
- **Module 2: Screening for HIV and Other Infectious Agents**
- **Module 3: Blood Group Serology.**

A module for trainers accompanies the set.

French, Spanish, Russian, Chinese, Portuguese and other language editions have also been produced. The English edition, first published in 1993, has recently been updated and reissued to reflect changes in transfusion medicine and laboratory technology.

_Establishing a Distance Learning Program in Blood Safety: A Guide for Program Coordinators_ provides a practical guide to the planning, implementation and evaluation of a distance learning program in blood safety.

**Additional WHO Learning Materials on Blood Safety**

Additional learning materials developed or being developed by the Department of Essential Health Technologies at the WHO include the following.

*Safe Blood Collection and Blood Components Production*

*Safe Blood and Blood Products* cover key aspects of the collection, testing and use of whole blood, but do not address the issue of blood components. In order to provide comprehensive guidance on the process of component production, two further guides—*Safe Blood Collection*, and *Blood Components Production*—are being developed.

**Clinical Use of Blood**

*The Clinical Use of Blood* consists of an open learning module and pocket handbook which provide comprehensive guidance on transfusion and alternatives to transfusion in the areas of general medicine, obstetrics, pediatrics and neonatology, surgery and anesthesia, trauma and acute surgery, and burns. The publications are designed to promote transfusion of the right blood to the right patient and appropriate clinical use including the wider use of plasma substitutes, more effective prevention and treatment of conditions that may make transfusion necessary and a reduction in unnecessary transfusions.

These materials have been developed for prescribers of blood as well as for blood transfusion personnel, particularly for staff at the first referral level in developing countries. They have been designed for use in undergraduate and postgraduate programs, in-service training and continuing medical education programs, including distance learning programs, as well as independent study. To further facilitate the use of these learning materials, the *Clinical Use of Blood* module and handbook are also available on CD-ROM.

The WHO has also published recommendations on *Developing a National Policy and Guidelines on the Clinical Use of Blood* which encourage the use of the learning materials in education and training programs to promote effective clinical decisions on transfusion.

**WHO Blood Cold Chain Project**

Access to and use of appropriate technology are essential for the safe cold storage and transportation of blood from donation to transfusion, a process referred to as the blood cold chain. The WHO *Blood Cold Chain Project* is meeting this challenge by providing technical and logistics information that will em-
power managers of health care programs to improve consistency in the management of the blood cold chain. A guide entitled *The Blood Cold Chain—Guide to the Selection and Procurement of Equipment and Accessories* provides the WHO minimum specifications for a wide variety of equipment required for an effective blood cold chain and the specifications for selected blood cold chain equipment evaluated by the WHO. Specific guidance in how to select and procure blood cold chain equipment and accessories is also provided.

**Promotion of Voluntary Blood Donation and World Blood Donor Day**

Pledge 25, a youth program initiated in Zimbabwe which has significantly contributed to the safety of the national blood supply, is an innovative approach to the recruitment and retention of young, voluntary non-remunerated blood donors from low-risk populations. The success of the Pledge 25 Club has generated interest in many developing countries and the approach was promoted as part of the activities of the World Health Day 2000. A similar program has been established in South Africa and pilot projects have been initiated in different regions to promote the concept. Training activities have been conducted in the regions to support Member-States in establishing voluntary blood donor programs. Training materials for the training of donor recruiters and blood donor managers are also being developed.

Building on the momentum of the World Health Day 2000, the WHO worked together with the International Federation of Red Cross and Red Crescent Societies, International Society of Blood Transfusion and International Federation of Blood Donor Organizations to launch the World Blood Donor Day on 14 June 2004. The World Blood Donor Day was celebrated on 14 June 2004 in nearly 80 countries. This was designed to raise awareness about the vital need for voluntary non-remunerated blood donations from low-risk populations, and to applaud the contribution made by blood donors.

**WHO Schemes for the Evaluation and Bulk Procurement of HIV Test Kits**

The WHO established the *HIV Test Kit Bulk Procurement Scheme* in 1989. The goals of the scheme are to facilitate access to high quality test kits at low cost through an easy purchasing procedure and to provide additional information and assistance to those selecting and purchasing test kits to ensure that the chosen kits will be appropriate for the conditions in which they will be used and will meet the overall testing objectives. The Bulk Procurement Scheme is directed towards and assists national HIV/AIDS control programs and BTSs. The scheme contributed to savings of around US$ 5 million in 1999 alone. Hepatitis B and C test kits will also be added to the Bulk Procurement Scheme. Recommendations and guidelines have been developed by the WHO to assist in the selection of appropriate kits.

**Hemoglobin Color Scale**

The WHO Hemoglobin Color Scale is a new, cost-effective device to screen for anemia which is rapid and inexpensive (less than US 2 cents per test). The clinical utility of the Scale has been demonstrated in the management of malaria, pregnancy, child and adolescent health care programs, anemia and iron therapy control programs, anthelminthic control and nutritional programs and the screening of blood donors to ensure their suitability to donate blood. The Scale is an invaluable tool for health care personnel at all levels of the health system where more expensive photometric methods are not readily available, especially at district and peripheral health services in developing countries.

**CONCLUSIONS**

Ensuring blood transfusion availability and safety is an achievable goal. The WHO has responded to requests by Member-States that it should take the lead in promoting blood transfusion safety. By further increasing its commitment in this area, the WHO fulfils its leadership role in the prevention of risk factors for major infectious disease burden and in organizing equitable health services in line with its strategic directions. These goals cannot be achieved without the commitment and support of Member-States. The WHO is committed to blood transfusion safety as one of its priorities and will continue to work with its partners—experts in blood transfusion, the WHO collaborating centers and other international institutions and organizations in blood safety—to develop effective strategies and mechanisms to achieve the objective of global blood safety. The overall
improvement in blood safety will lead to a decrease in the risks associated with blood transfusion, notably a reduction in the morbidity and mortality associated with transfusion and a reduction in transfusion-transmitted infection, and will contribute substantially to the decrease in the global burden of disease.

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The goal of any transfusion service is to provide blood components that are safe for transfusion and that pose minimal risk of transfusion-transmissible infections. To achieve maximum safety at an acceptable cost requires a multilayered risk reduction strategy involving safe blood donors, safe blood components and safe transfusion practices. Obtaining safe blood donors who have a low risk of carrying transfusion transmissible infections requires effective donor selection and screening strategies. Safe blood depends also on laboratory testing with stringent quality controls and processing to inactivate infectious agents or remove of infectious cells in the blood components. Safe transfusion practices are essential so the correct blood is given to the correct patient and given only when it is truly needed.

As a first step to achieving this goal, The National Blood Transfusion Council, India in the year 2002 formulated the “Blood Policy” and the “Action Plan” to implement this policy. The highlights of these recommendations include Rationalization of Blood Transfusion Service (BTS); achieving 100 percent blood collection from voluntary non-remunerated blood donors; and institution of quality systems in blood centers, among others.

Rationalization of BTS can be effected by a tiered system of identified Regional Blood Transfusion Centers (RBTCs) which collect, test and process blood into components for issue to hospitals’ blood storage centers. This makes implementing and monitoring of quality systems easier and more effective. It also results in better utilization of available resources and, therefore, makes for better economic sense. It makes for easier access to blood components for the end user.

Thus, the ideal roles and responsibilities of a Regional Blood Transfusion Center (RBTC) are to:

• Cater to the blood needs of the population in its assigned area of responsibility.
• Have the ability to cater to blood needs in emergencies and disasters.
• Provide round-the-clock service.
• Have a well-organized program for promoting voluntary blood donation and donor retention.
• Have a voluntary donor base that is adequate to meet the requirement for blood in the region.
• Have facilities for conducting blood donation drives (blood donation camps) and for transportation of the collected blood to the RBTC.
• Have in place a quality management system and ensure good manufacturing/laboratory practice
• Carry out the mandated screening tests for transfusion-transmissible infections on all the blood units collected.
• Have a mechanism to notify blood donors found to have positive tests for infectious diseases, such as hepatitis, HIV, etc. and other clinically significant abnormalities detected during the predonation evaluation or during laboratory testing. This should include notification, counseling and referral services for donors whose samples test positive for infectious diseases.
• Process and store the blood collected into components.
• Have a well-organized system for issue and transport of blood and components to the blood storage centers at the hospitals it caters to.
• Be a regional reference laboratory for red cell serology testing, capable of identifying unexpected red cell antibodies, identifying compatible units and handling typing and compatibility problems.
• Offer consultative service to the end-users and handle technical and clinical problems.
• Audit blood use and take necessary action to improve transfusion practice in hospitals.
• Have automated laboratory-testing procedures to facilitate a computerized management information system.
• Have the necessary financial resources and infrastructure in terms of premises, personnel and equipment.
• Be accredited by a national and international accreditation agencies.
• Take part voluntarily in blood-use audits, hemovigilance and other look-back programs.

In addition to the basic services, an RBTC should:
• Provide the relevant educational and training programs for clinicians, BTS personnel and the community on transfusion medicine and related topics and provide technical know-how for other blood centers.
• Network with other blood centers, hospitals, laboratories, academic and research institutes, etc. in order to provide the best service and also constantly upgrade the quality of services provided.
• Undertake research in key areas related to transfusion medicine, immunohematology and blood safety.
• The RBTC also has an important role in advocacy with the government, community and other stakeholders.

An RBTC can also provide other services such as red cell panels and plasma products; transplant immunology (HLA) laboratory; stem cell and umbilical cord blood collection and cryopreservation; other laboratory services for hematological disorders such as thalassemia, hemophilia, leukemia, etc.

ORGANIZATION OF A REGIONAL BLOOD TRANSFUSION CENTER

Legal and Regulatory Authority

Blood banks in India come under the licensing, regulatory authority and oversight of the Drug Control Department, Government of India. They have to comply with the minimum standards laid down by the Drugs and Cosmetic Act, 1940 for the requirements for suitable space, environment, equipment, staff and the mandated tests to be carried out in order to maintain safe and acceptable standards in recruitment and selection of blood donors, collection, testing, processing, storage, distribution and transfusion of blood and blood components. It also mandates that the blood bank shall establish a program for quality control and quality assurance and specifies the documentation requirements including a quality manual, standard operating procedures (SOPs) and all relevant records.

Planning for a Regional Blood Transfusion Center

Setting up or upgrading an existing blood center into an RBTC, should be based on a needs assessment study to assess the blood needs of the community. This includes, among other things, the population demographics; types of medical facilities and services available (tertiary or trauma care facilities will need more transfusion support); estimates of current and future transfusion practice and requirement of blood components; existing and emergent disease patterns; the potential in the community for volunteer blood donors and so on.

Based on this, a detailed project proposal will have to be drawn up which should include the infrastructure (building, equipment and staffing) requirement; appropriate technology; estimated cost and budgets; funding plan; logistics of blood procurement, testing, processing and issue of blood components; legal and licensing requirements; and a time line for the action plan.

The stakeholders for an RBTC include the blood donors, voluntary organizations and volunteers; the media and advertising and communication agencies and experts; blood users (hospitals, clinicians, patients
and other blood centers); blood center staff; service providers (electricity, water and sewerage, biowaste disposal); manufacturers and distributors of equipment and consumables; scientific experts in blood banking and immunohematology; legal and ethical experts; external reference laboratories, accreditation agencies; governmental health departments; regulatory authorities (Drug Control Department); funding agencies and so on.

**Infrastructure Requirements**

**Building**

There should be sufficient space, facilities and environment conducive to ensure optimum and efficient performance of the work and provide satisfactory service. Adequate lighting; ventilation including fume cupboards, air conditioner and biohazard cabinets; and regular as well as emergency power and voltage stabilizers to take care of electrical power fluctuations should be ensured.

**Equipment and Spares**

Adequate and appropriate equipment should be available for the collection, testing, processing, storage and issue of blood and the staff trained and competent to operate them. Purchase of equipment should be based on the specifications that should be met; installed by the authorized agency and a certificate of satisfactory installation signed only after thorough check on proper functioning of the equipment is made. A schedule for preventive maintenance, calibration and validation should be prepared in consultation with the dealer and a maintenance contract entered into for all major equipment. Consideration of equipment should include names and details of the manufacturer, supplier, power requirement; QC, calibration, validation, cost, import issues, etc.

**Staffing**

There should be sufficient professional and support staff with the required educational qualification, training and experience as laid down by the Drug Control rules. The RBTC should be under the direction of a licensed physician, with a degree in Medicine (MBBS) and postgraduate training in pathology, transfusion medicine and who shall have the responsibility and authority for all medical and technical policies and procedures; and for the supportive services that relate to the safety donors, blood components and patients. The Medical Officer is also responsible to notify the donors of all clinically significant abnormalities detected during the pre-donation evaluation or laboratory testing and be involved with evaluating reports of adverse reactions in transfusion recipients.

Other staff includes technicians with a diploma in medical laboratory technology; technical supervisors; Quality Control Officer; registered nurses; social workers, motivators and administrative staff. All personnel, especially the technical staff should undergo an orientation and position description at the time of joining and regular training, continued education programs and periodic assessment. Work assignments should be consistent with the education, training and experience of the staff. BTS staff should be trained to be helpful, cooperative and empathetic with donors as well as patients. The work ethic that “The customer is always right” works for BTS as it does for any other service organization.

The RBTC should have a panel of experts as external advisors. It should also provide consultative service to clinicians for advice on the precision and accuracy of methods used in the blood bank; the statistical significance of results and their relation to reference ranges; the scientific basis and the clinical significance of the results; the suitability of the requested procedure to solve the clinical problem; and the further procedures which may be helpful.

The basic areas of operation in an RBTC include donor recruitment, eligibility determination, blood collection, red cell serology testing, testing for transfusion-transmissible infections, component preparation and storage; component issuance and transportation, quality control and quality assurance and transfusion facilities.

The necessary support systems include biosafety; inventory management; administration, housekeeping, management, finance and funding, counseling, public relations, etc.

**DONOR RECRUITMENT AND BLOOD COLLECTION**

The most important stakeholder for the BTS is the blood donor. The various categories of blood donors
include the voluntary non-remunerated donor, the paid donor, the replacement donor, the directed donor, the designated donor and the autologous donor. The voluntary non-remunerated donors give blood with the purely altruistic motive of helping an unknown patient and not for payment or any favors. They respond readily to appeals for blood donation and donate blood regularly. Since they are not under duress to donate, they give more reliable information at the time of donor screening and self-defer if there has been any behavior placing them at risk of contracting hepatitis, human immunodeficiency virus (HIV) or other transmissible disease. Therefore, the incidence of transfusion-transmissible infections is very low in this donor group. Paid donors give blood in return for payment or other favors. The worldwide experience indicates a high incidence of transfusion-associated infections among them. Paid donations have been banned in India since 1998 as in most other countries. Replacement donors “replace” the blood issued by the blood center to their relatives or friends. Since they may be under various types of pressure or inducement to donate by their relatives or friends who have recently been transfused, they may be less truthful when asked screening questions or less likely to self-defer, and thus compromise blood safety. Directed donors give blood prospectively to a specific, named patient who will be needing blood available. This should be avoided among close relatives or the donation must be subjected to irradiation since donations from close relatives can lead to the rare, but invariably fatal, transfusion-associated graft-versus-host disease (GVHD). Designated donors take care of a particular patient’s recurrent blood requirement. Autologous donors donate blood for their own use. This ensures safety for them and also helps to conserve precious bloodstocks for other patients.

Recruitment of Voluntary Donors

Donor recruitment is inducting suitable persons to donate blood. People have to be motivated to become blood donors. The ability to motivate is a skill that can be taught. Motivation programs use various strategies and different media to educate and motivate potential donor groups. They have to be ongoing and sustained to be effective. Surveys done worldwide indicate that young people are easier to motivate and demonstration of blood donation by peers is a good motivation factor. Very often, the reason why people do not donate is because they did not know that it was needed—“Nobody asked me” is a common refrain that has to be tackled with an effective awareness program. Making the blood donation process as convenient and easy as possible by having mobile blood donation camps at the workplace or universities increases collections.

Blood Donor Retention

Studies have shown that blood obtained from regular non-remunerated voluntary blood donors show fewer positive results for infectious diseases than blood obtained from the first-time and replacement blood donors. Regular, repeat blood donors enhance blood safety. Use of these donors involves less time, money and effort than recruiting new blood donors.

A safe and pleasant first-time donation experience with good donor care promotes repeat donation. Appreciation and recognition shown publicly with presentation of small mementoes like certificates, badges are helpful. Being in touch with donors through birthday or anniversary messages or newsletters, continuous efforts to improve services, confidence and trust in the BTS, support for the donor’s own need for blood and getting and acting on donor feedback and complaints are also effective donor retention strategies.

Often, even regular donors stop donating and are called “lapsed donors”. This may be because of medical reasons, dissatisfaction with the BTS due to poor donor care or not getting timely help when they need blood, inconvenient time or place to donate and so on. The reasons for the lapse should be established and as far as possible rectified.

Blood Donor Screening and Selection

Predonation screening for medical fitness is for donor safety as well as to ensure that the blood gives maximum benefit and carries minimum risks associated with transfusion. The first step in this is donor registration to get demographic and contact infor-
mation to enable proper identification, notification, and for possible traceability and recall purposes.

Next, the potential donor is given “predonation information” about deferrable risk behavior for transfusion-transmissible infections. This along with the risk behavior questionnaire that is administered, and the predonation counseling, provides the opportunity for self-deferral. The questionnaire is also an important legal document that has the informed, signed consent for blood donation and screening for infectious markers.

A medical history and examination by qualified BTS staff to determine suitability to donate is based on donor eligibility and deferral criteria. These criteria may differ in different countries. In India, the criteria should be as per the Drug Control Rules.¹

Unfortunately, deferrable risk behavior screening procedures in India are not uniform and a very few centers have a risk behavior questionnaire. Lack of sufficient numbers of trained counselors; not knowing the importance of risk behavior screening; and the embarrassment associated with discussions on sexual risk behavior are some reasons why this is so.

Blood Collection

Blood should be collected by aseptic methods using sterile blood bags by a single venipuncture. The standard operating procedure (SOP) for blood collection should include the details for the preparation of phlebotomy site; labeling; venipuncture, collection of blood and test samples; transport, postdonation care and management of donor reaction. All containers and anticoagulants used for storage and preservation of blood and blood components and all reagents used for blood samples should meet the standards of Drugs and Cosmetics Act of India.

The blood bags should be labeled with the unit number, blood group, date of collection, etc. prior to collection. The preparation of the phlebotomy site is important to prevent bacterial contamination of the blood. Blood is collected in 350 ml single blood bag or in a 450 ml, multiple bags, with 14 ml anticoagulant solution per 100 ml blood collections. The proper and gentle mixing of the blood with the anticoagulant solution should be ensured. Separate pilot samples should be collected for laboratory tests. After collection, the blood bag tubing should be sealed and cut and the needle with any attached tubing disposed as bio-hazard waste.

The phlebotomy site should be covered with a sterile dressing after blood flow ceases. The donor should be given some light refreshment and kept under observation for fifteen to twenty minutes for any signs of adverse reactions. The collected blood should be stored in a separate quarantined area prior to screening for transfusion-transmissible infections (TTI) at 4 degrees Centigrade except for blood meant for platelet preparation, when it is stored at 22 degrees Centigrade.

RED CELL SEROLOGY TESTING

Routine red cell serological testing includes ABO blood group and Rh typing of the donor including subgroups such as A2, Bombay O and Weak (Rh) D when necessary. Both cell and serum grouping should be done by tube or microtiter plate methods. Donors and recipients are tested for unexpected red cell antibodies using albumin or antihuman globulin (AHG) methods. Identification of the unexpected antibodies requires panel cells and is carried out at specialized immunohematology centers. For patients, a pretransfusion crossmatch using patient plasma and donor red cells for red cell and whole blood transfusions. This can be performed either by the conventional tube method or by the newer column-agglutination or bead technologies.

A transfusion reaction investigation should include a post-transfusion blood sample tested for the direct antiglobulin test and a determination visually whether the plasma is red or pink.

Red cell serological testing requires three sets of reagents; those for ABO grouping, Rh (Rhesus) typing and antiglobulin reagents. Monoclonal reagents, prepared from hybridomas, are more specific; have high reaction speed and titer; are stable and cost effective and are preferred to polyclonal reagents, which are expensive and variable in quality. Monoclonal complement antibodies can be used as a blend with polyclonal AHG reagents. It is preferable to always keep antisera of two different manufacturers or different batches from the same manufacturer in stock. All reagents should contain a preservative to minimize bacterial and fungal growth and should be
clearly labeled with the batch and lot number; date of expiry, storage temperature, name of manufacturer and the preservatives used. They should be stored in a refrigerator at 4 to 6°C and always used according to manufacturer’s instructions or re-standardized if they are to be used by alternate techniques or in a diluted form.

Quality Control of Blood Group Reagents and Cells

The quality control (QC) of reagents should detect deviation from the established minimal quality requirements as laid down by national and international standards. Any deviations from these standards should be reported to the QC officer and manufacturer. A quality evaluation should be performed on samples before purchasing larger batches of commercial reagents to ensure that they meet specifications, minimal quality requirements and biological standards. In addition, cell grouping and serum grouping performed daily acts as a built-in quality check for ABO reagents.

The QC checks test for specificity, sensitivity and avidity. Testing sensitivity involves the ability of the test to detect reactions between homologous antigens and antibodies, which are specific to the corresponding antigen and antibody. The specific antiserum should react with the corresponding cells only. For example, anti-A reacts only with A cells and not with B and O cells. Testing sensitivity involves the reciprocal of the highest dilution or the lowest concentration, which will give visible agglutination and is determined by testing two-fold serial dilution of the antiserum in saline against selected red cells. Testing avidity involves the strength of the bond after the formation of the antigen-antibody complex.

Reagent red cells that are available commercially are expensive. Most blood centers, therefore, prefer to prepare them in-house from known donor samples. These should also undergo quality checks and thereafter can be refrigerated and used for one week. The immunoglobulin G (IgG)-sensitized Coombs’ control cells prepared fresh every morning are used to validate every test in which AHG is used. Addition of control cells to a negative antiglobulin test should produce agglutination.

REDUCING THE RISK OF TRANSFUSION-TRANSMISSIBLE INFECTIONS

Blood transfusion is an important part of modern medicine and often a life-saving procedure. But it carries the major risk of transmission of infections because a large volume of human source material is infused directly into the body and blood collected from a single infectious donor may be transfused to a large number of recipients. Transfusion-transmissible agents have certain characteristics. They are present in an infectious or potentially infectious form in blood; are stable during storage and transmitted via the parenteral route. Most importantly, they can cause asymptomatic infections in the donor and an apparently healthy prospective donor could potentially be harboring infections.

To minimize transmission of these diseases, three levels of safety strategies are instituted. Level one is the predonation screening to defer unsuitable donors with risk behaviors. Level two includes screening of the donated unit for the presence of infectious disease markers, and level three involves minimizing blood transfusion to the extent possible and using blood only when truly needed.

A number of viruses, bacteria, protozoa and prions fall into the above category including, hepatitis B and C, HIV-1, HIV-2, human T-cell leukemia virus (HTLV-I), HTLV-II, cytomegalovirus (CMV), Parvovirus –B19; Treponema pallidum, Salmonella, Brucella; Plasmodium species, Toxoplasma and Trypanosoma; variant-CJD (Creutzfeldt-Jakob disease) and so on. In India, as per the Drug Control mandate, every unit of blood collected should be screened for antibodies to syphilis; HIV-1 and 2; and hepatitis C virus; hepatitis B surface antigen (HBsAg) and for the malarial parasite. Only units found non-reactive to all mandated tests should be released for issue. All reactive units are to be disposed of as biohazardous waste.

Treponema pallidum causes syphilis, a sexually transmitted disease. The first symptom appears within 9 to 15 days after exposure as a small ulcer on the sexual organs, which often goes unnoticed because it is painless. Treponema pallidum antibodies are present throughout life.

The flocculation test for treponemal antibodies uses cardiolipin as the antigen [Venereal Disease Research
Laboratory (VDRL) or RPR] and is nonspecific as cardiolipin is a normal tissue component and 1 percent of normal adults produce non-specific antibodies to it. The *Treponema pallidum* hemagglutination (TPHA) and enzyme-linked immunosorbent assay (ELISA) are more specific.

**Malarial Parasite**

The four species of *Plasmodium* that cause malaria are *P. vivax*, *P. falciparum*, *P. ovale* and *P. malariae*. The life cycle takes place in two hosts, the salivary glands of mosquito and red cells and the liver cells in man. Screening for malaria is by microscopic examination of thick films of peripheral blood smears with Geimsa stain. The appearance in peripheral blood of the parasite gives rise to fever and chills. Since donors with history of fever are deferred, this test is usually negative. Donors giving a history of malarial infection should be deferred for a period of three years.

**Human Immunodeficiency Virus**

Human immunodeficiency virus (HIV), a retrovirus, causes the acquired immunodeficiency syndrome (AIDS) by slow destruction of the immune system. During the initial several years of HIV infection stage, the person remains outwardly healthy and symptom free. During the AIDS stage, the patient presents with signs and symptoms of opportunistic infections resulting from the immuno-compromised state.

HIV-1 and HIV-2 are two major, distinct types with significant, but not complete cross reactivity.

Screening tests for HIV-1 and 2 is currently based on serological detection of antibody to HIV-1 and HIV-2 by indirect, sandwich ELISA.

**Hepatitis B Virus**

Infection with hepatitis B virus (HBV) causes an acute, self-limiting or a chronic infection which can result in chronic hepatitis in 5 percent of cases; of whom, 20 percent go on to liver cirrhosis. Large amounts of noninfectious surface antigen and infectious virions (Dane particles) are produced and circulate in the blood of both acutely infected persons and the blood of chronic carriers, many of whom are without symptoms. The infection is often asymptomatic with some patients clearing the virus, while others remain carriers. A specific neutralizing antibody is produced during resolution of infection.

There is a sequential appearance and disappearance of serological markers during the infection—the surface, core and envelope antigens and the corresponding antibodies. HBsAg, the surface antigen, is the first and major marker, indicative of active acute or chronic infection and is produced in very large quantities. Anti-HBs is the circulating antibody indicative of immunity. Screening for HBV is by serological detection of HBsAg using sandwich ELISA. Rapid screening is based on detection of the HBsAg using immunochromatic tests.

**Hepatitis C Virus**

Acute hepatitis C infection is usually mild and often unnoticed except for mild icterus. Fifty to eighty percent of all infections progress to chronic infection with ten to twenty percent of these infections resulting in liver cirrhosis with or without hepatocellular carcinoma appearing after 20 to 30 years. Screening for hepatitis C virus (HCV) is by detection of anti-HCV antibody by indirect sandwich ELISA, using recombinant or synthetic antigens derived from nucleocapsid region (c22-3) and non-structural genomic regions of NS 3/4 and NS 5. Confirmation tests are still not ideal. HCV indeterminate and non-specific reactions are still reported as true positives. Seroreversion has been reported with apparent loss of antibody some time after resolution of acute infection and, therefore, can result in false-negative results. Rapid screening is based on serological detection of the hepatitis C antibody using immunochromatic tests.

**Principle of Indirect Sandwich ELISA for Donor Screening Tests**

In the indirect sandwich ELISA, the antibody present in the donor blood specimen binds to the antigen bound to a solid phase (microtiter wells, beads or nitrocellulose paper). When an enzyme-labeled synthetic antigen conjugate is added, it forms an antigen-antibody-antigen “sandwich”. This reaction is highly sensitive and specific. Detection is by addition of a substrate plus chromogen, which reacts with the enzyme and produces a color change that is
read by a spectrophotometer at a particular wavelength. Under standard conditions, the intensity of the color is proportional to the amount of specific antibody present in the test specimen. In the case of HBsAg, the surface antigen binds to the antibody on the solid phase.

Every kit has instructions for addition of reagents; incubation, washing, etc. and the specific number of negative and positive internal kit controls, that are to be assayed with each run. The cut off (CO) value is calculated from the absorbance values or optical density (OD) of the controls, by a formula, which is specific for each kit.

All specimens with OD lower than the CO are considered non-reactive and, therefore, fit for transfusion.

All specimens with OD higher than the CO are considered reactive, and not fit for transfusion.

Quality Control of ELISA tests include the use of test controls in each ELISA assay to monitor lot-to-lot variation; inter-run and intra-run reproducibility of the assay. The internal controls are the positive and negative controls that come with the kit. In addition, an external, positive QC sample should be assayed with each run. This can be a commercially available standard or one prepared in-house, by serial dilution of known positive samples, to give an OD closest to but not less than the cut-off.

The mean ratio of the OD of QC sample v/s that of the CO for first 15 to 20 runs is used to calculate the standard deviation as acceptable for the kit. Thereafter, the (QC) OD v/s CO ratio is plotted in a graph for every run and should lie between two standard deviations for the run to be valid. Changes, shifts and trends in the graph are also indicative of problems in the reagents.

Precautions to prevent contamination from carry over of one sample well to another and minimize errors include the use of new, disposable pipette tip for each sample. In addition, test kits should not be used beyond expiry and reagents from different lots should not be mixed or interchanged. The instructions as per kit insert should be strictly adhered to. Repeated freeze-thaw and heating to 56°C should be avoided as the serum can get inactivated and give non-specific results. The test samples should be stored at 4°C pending testing as well as for seven days afterwards.

Rapid assays combine the simplicity of particle agglutination with ELISA technology. The antigen or antibody is immobilized on either a porous or a semi-porous membrane or strip to which the test sample and conjugate are added. The result is read visually. Drawbacks of ELISA tests include false-negative results, especially during the seronegative window period prior to development of the antibody and non-specific false-positive results.

The first consideration for choice of test kits is its sensitivity and specificity. Other factors include ease of performance, equipment required; cost; volume of work, etc. Sensitivity is the ability of the test to detect the weakest sample possible. Tests with high sensitivity will give fewer false-negative results and are, therefore, important for transfusion and transplant safety. Specificity is the ability of a test not to detect false or non-specific positives. Tests with high specificity are important for diagnosis.

The WHO recommended HIV testing strategy for transfusion safety is as follows. Initial screening should be performed with a highly sensitive combined HIV-1 and HIV-2 assay. All samples with CO greater than OD should be retested with the same kit, in duplicate, with a sample from the blood bag tubing. This is necessary to take care of errors in sampling, identification, spillage, carry-over and so on. If one or more of the repeat tests show a positive reaction, the sample (blood unit) is labeled initial reactive (IR). All IRs or indeterminate donations should be discarded as biohazardous waste.

If donors are to be notified, the IR units are tested with a second assay based on a different antigen preparation or different test principle. A repeatedly reactive (RR) result is considered positive for HIV.

If the second test is negative, it is tested with two more assays. Concordant results will determine whether the sample is positive or negative. Samples with discordant results are termed indeterminate.

The residual risk of transmission of infectious diseases even after screening in low incidence, low prevalence countries with developed health care systems, is mainly from ‘window period’ donations. Residual risk in high incidence, high prevalence countries, very often also with poorly developed health care systems, is from a combination of lack of testing, testing errors, and ‘window period’ donations. Strategies for screening for other infectious disease
markers such as direct pathogen testing for HIV and HCV nucleic acid are being adopted by some countries to reduce residual risks.

The records of donor infectious disease screening results should be maintained for five years and the reports of positive results submitted to the Drug Control Department, Government of India as well as the National AIDS Control Organization. The RBTC should maintain a registry of donors deferred for positive infectious disease tests to ensure that anyone deferred permanently does not donate by checking the registry for names of prospective donors and deferring them if they are on the list.

**Notification of Donors with Reactive Test Results**

Until recently, in India, there was no system of donor notification for reactive infectious disease test results. As of December 2002, it was decided that donors who give informed consent for this should be notified. A counselor with pre- and post-test counseling training should perform this. Maintaining confidentiality is of the utmost importance, especially in the case of HIV because of the stigma attached to HIV infection.

**Preparation, Storage and Use of Blood Components**

Blood transfusions are required to maintain adequate oxygenation of tissues, treat bleeding and coagulation disorders, correct immunological deficiency or to maintain blood volume. Whole blood is indicated in very few, restricted clinical settings where red cell and blood volume deficit are simultaneously present. It is also indicated in exchange transfusion for neonates. In all other cases, specific components to correct the particular deficiency should be given. By transfusing red cells instead of whole blood, other components such as plasma, platelets and cryoprecipitates can be available for other patients and the transfusion of unneeded components, such as plasma or platelets, is avoided reducing the risk of volume overload or other complications from those components.

**COMPONENT PREPARATION FROM WHOLE-BLOOD DONATIONS**

Blood components are prepared by centrifugation of whole blood in a refrigerated centrifuge, which results in sedimentation of the various blood components into the different layers depending on their size and density. Thus, the red cells are at the bottom, leukocytes at the top of the red cell mass; next, the platelets; and lastly, the plasma in the upper part of the bag. After the first centrifugation, the primary bag is placed in a plasma extraction system and the layers are transferred, one by one, into satellite bags within the closed system. For platelet preparations, centrifugation is done at 22°C and for all other products at 4°C. Red cell units are stored at 4°C. Platelet-rich plasma can be further centrifuged to prepare platelet concentrates to be stored at room temperature and plasma stored in a frozen condition.

Blood components thus prepared are stored in conditions designed to preserve optimal viability and function (Table 3.1). Storage equipment must have surplus capacity, uniform temperature distribution within the unit and temperature recorders and alarms. They must be easy to inspect and clean and conform to local safety requirements. A reserve power supply is also a must to take care of power shortages. Separate storage space should be reserved and clearly indicated for units kept in quarantine awaiting completion of testing.

**Table 3.1: Blood components prepared from whole blood**

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage temperature</th>
<th>Storage period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood, red cells in CPDA</td>
<td>2 to 6°C</td>
<td>35 days</td>
</tr>
<tr>
<td>Whole blood, red cells in additive solution</td>
<td>2 to 6°C</td>
<td>42 days</td>
</tr>
<tr>
<td>Platelet concentrates</td>
<td>20 to 24°C with agitation</td>
<td>5 days</td>
</tr>
<tr>
<td>Frozen plasma, cryoprecipitates</td>
<td>(–) 18°C or below</td>
<td>1 year</td>
</tr>
<tr>
<td>Cryo-poor plasma</td>
<td>(–) 18°C or below</td>
<td>5 years</td>
</tr>
<tr>
<td>Frozen red cells</td>
<td>(–) 80°C or below</td>
<td>&gt;10 years</td>
</tr>
</tbody>
</table>

**QC in Blood Component Preparation**

**Donor Selection and Blood Collection**

Other than the general principles and measures to be followed for donor screening and collection of whole blood, certain additional and specific conditions have to be met when separating whole blood into components. The body weight of the donor should be more
than 50 kg. Aspirin or aspirin-containing compounds depress platelet function for 1 to 5 days. Therefore, blood collected from a donor with aspirin intake within five days prior to donation should not be the only source of platelet concentrates for a particular patient.

Approximately 450 ml of blood is collected in a sterile pyrogen-free multiple plastic bag system with integral tubing in a closed system. To prevent partial activation of the coagulation system, the blood must be collected with a single venipuncture and with minimal trauma to the tissues. A second clean venipuncture with a new needle at a separate site is acceptable. Sufficient and uninterrupted flow of blood should be ensured as the bag fills. Donation of one unit of whole blood ideally should not be more than 10 minutes. If the duration is more than 15 minutes, the plasma should not be used for preparation of platelets and plasma. Frequent gentle mixing, preferably with an automated system, is a must to ensure proper mixing of the blood with the anticoagulant during the donation. Immediately after collection, the tubing should be sealed, preferably with a heat sealer. All satellite bags must be accurately identified, numbered and labeled as on the original unit.

The blood must be processed for component production within eight hours of collection. Meanwhile, it should be stored at 2 to 6°C except for preparation of platelets and plasma. Frequent gentle mixing, preferably with an automated system, is a must to ensure proper mixing of the blood with the anticoagulant during the donation. Immediately after collection, the tubing should be sealed, preferably with a heat sealer. All satellite bags must be accurately identified, numbered and labeled as on the original unit.

Calibration of Blood Bank Refrigerated Centrifuges

Centrifuges used for producing components can have different rotor sizes and other variables and should be calibrated for the optimum centrifugation speeds and time, upon receipt as well as after adjustments or repairs. This is done by comparison of the QC of components prepared at several different speeds and time and choosing the one that gives the best component. Automatic electronic braking systems ensure prompt deceleration and minimum re-suspension of centrifuged cells. To ensure safety, there should be mechanisms to ensure that the centrifuge cover cannot be opened till the rotor comes to a complete stop and overwraps may be used to contain spills that may occur due to leaks or rupture of the bags or tubes. To ensure better separation of cells, the bag should be kept with the primary bag farthest from the center, with the broad side facing outwards. Weight distribution in opposing cups must be equalized with balancing material like rubber disks to prevent damage to the rotors and improve efficiency. Swing out cups provide better separation than fixed-angle cups.

Optimal Preparation of Plasma Components

Freezing is a critical step in the preservation of frozen with optimal levels of the plasma coagulation factor VIII. At slow freezing rates, water freezes into pure ice in the periphery, while the plasma solutes get displaced to the middle of the plasma unit and can crystallize. Factor VIII molecules exposed to concentrated salts for prolonged periods during slow freezing rates lose procoagulant functional activity. At more rapid freezing rates, water freezes more uniformly with clusters of solute crystals more homogeneously trapped in the ice. Factor VIII, not in prolonged contact with concentrated salts, has better preserved functional activity. Therefore, to achieve high yields of factor VIII, plasma should be frozen to below minus 30°C within one hour by placing the units in dry ice-ethanol or dry ice-antifreeze bath, or between layers of dry ice or in a blast freezer. Frozen units should be handled with care since the bags are brittle. Any bags that leak must be discarded.

Various methods can be used to detect inadvertent thawing. One method is freezing the plasma in a horizontal position but storing it upright. A movement of air bubbles will indicate that it has been thawed. Using another method, the tubing that is pressed into the bag during freezing forms an indentation which will disappear if the unit thaws. These simple procedures can help detect if there has been unsuspected thawing and refreezing of plasma, especially during transportation.

Quality Control of Blood Components

A representative sample (e.g. 1%) of blood components prepared is evaluated for the specified amount
of component or level of activity (Table 3.2). If 75 percent of the units tested meet the quality requirement (QR), it is assumed that similarly processed units are of equal quality and suitable for transfusion. When test results are unacceptable, an investigation of the cause and corrective action is to be undertaken and recorded.

<table>
<thead>
<tr>
<th>Blood component</th>
<th>Parameter</th>
<th>Quality requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell concentrate</td>
<td>Hemoglobin</td>
<td>Minimum 45 g per unit</td>
</tr>
<tr>
<td>PCV (HCT)</td>
<td>0.65 to 0.75</td>
<td></td>
</tr>
<tr>
<td>Random donor platelet concentrate</td>
<td>Platelet content</td>
<td>&gt;60 × 10^9 per unit</td>
</tr>
<tr>
<td>Single donor platelet, pheresis</td>
<td>Platelet content</td>
<td>&gt;200 × 10^9 per unit</td>
</tr>
<tr>
<td>Plasma</td>
<td>pH</td>
<td>6.5-7.4</td>
</tr>
<tr>
<td></td>
<td>Factor VIII-C</td>
<td>&gt;0.7 IU per ml</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>200-400 mg per unit</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>Factor VIII-C</td>
<td>&gt;70 IU per unit</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>150-200 mg per unit</td>
</tr>
</tbody>
</table>

**Table 3.2: Quality control of blood components**

**Irradiated Cellular Blood Components**

Viable lymphocytes in blood components can cause a rare but nearly always fatal, post-transfusion graft-versus-host disease in immune compromised patients or if transfused with HLA-matched components between family members or in genetically homogeneous populations. Giving red cell products treated with 25 to 40 gray (Gy) of ionizing radiation prevents this.

**BLOOD COMPONENT PREPARATION BY APHERESIS**

Preparation of components by apheresis uses centrifugation or filtration techniques. The component, most often prepared by apheresis, is single donor platelets. For this, as the blood is drawn from the donor, it is mixed with anticoagulant solution and pumped into a rotating bowl, chamber or tubular rotor. The cells get separated into different layers and are diverted, while the rest of the blood is returned to the donor. Depending on the method of preparation and the machine used, the platelet yield per procedure will vary from 200 to 800 × 10^9. A standard unit of apheresis-collected platelets should be the equivalent of a pool of five random platelet concentrates obtained from whole-blood donations.

Plasma can be donated by plasmapheresis, whereby plasma is collected by using filtration or centrifugation blood processors on healthy donors. These same blood processors can be used during plasmapheresis for therapeutic purposes when abnormal plasma constituents are needed to be removed in a patient and replaced with plasma of albumin. There are two basic types of apheresis blood processing equipment. The intermittent-flow type where the centrifuge bowl is alternatively filled and emptied uses one intravenous access line to draw and return the blood. In the continuous flow method, two intravenous access lines are used, one for removal and the other for return of blood.
Apheresis staff, who operate the blood processors, must be adequately trained and under the supervision of a trained physician. Donors with history of abnormal bleeding episode or an adverse reaction during previous donations should be deferred. For platelet pheresis donations, the platelet count of the donor should be > 150 × 10^9 per ml and donors with history of aspirin intake within the previous five days should be deferred.

The volume of extracorporeal blood in any apheresis procedure should not exceed 13 percent of the donor’s estimated blood volume.

**Monitoring Apheresis Donor Eligibility**

The interval between one whole-blood donation and the next apheresis donation should be at least one month. The interval between apheresis and a subsequent whole blood donation should be at least 48 hours. Donors should not undergo plasmapheresis more than once in two weeks and should not give more than 650 ml of plasma per donation or more than 15 liters of plasma per year. Erythrocyte loss per donation should be less than 20 ml of packed cells per week. A qualified physician should evaluate serial plasmapheresis donors at least once a year, including serum protein estimation.

**ISSUE AND TRANSPORTATION OF BLOOD COMPONENTS**

A validated system should exist to ensure that blood components are maintained at recommended storage temperatures during transport, if possible with some form of indicator to monitor the in-transit temperature. The containers used in transport should be well insulated, easy to clean and easy to handle. Where a dedicated refrigerated vehicle is used, the principles applying to control of the refrigerators should be observed. Alternatively, systems for air, road or rail transport using controlled cool may be considered. These coolants must not come into close contact with the blood bags. Brief information about the blood component should be made available to clinicians and transfusionists with regard to definitions, composition, indications, storage and transfusion practices.

Returned blood components should not be reissued for transfusion if the bag has been penetrated or entered, the product not maintained continuously within the approved temperature range or if there is evidence of leakage, abnormal color change or excess hemolysis. The proper identification, time of issue and transit history should be fully documented.

**AUTOLOGOUS BLOOD TRANSFUSION**

Clinicians should be encouraged by the RBTC to perform autologous blood transfusions as they avoid the risks of alloimmune complications of blood transfusion, and reduce the risk of transfusion-associated infectious complications. Autologous blood components can be obtained by preoperative donations, by acute normovolemic hemodilution or by intraoperative collection of blood shed during surgery. Preoperative autologous donations of whole blood or components can take place weekly preceding elective surgery. Preoperative autologist donation may be carried out safely even in elderly patients, children over 10 kg body weight, and in patients who have medical illnesses such as stable cardiac disease, etc. Usually, the anesthesiologist or the surgeon may determine the suitability of drawing blood from the patient and then prescribe preoperative donations along with oral iron supplements.

Autologous blood units should be collected, prepared and stored in blood transfusion centers and include the routine donor infectious disease testing and compatibility tests, using the same protocols as for allogeneic donations. Presurgical autologous blood components should have a distinct label and issue procedures must include a confirmation of identity written on the component labels, on the prescription document and at the bedside. Untransfused autologous blood components must not be used for allogeneic transfusions without previous informed, written and signed consent from the autologous donor.

Acute normovolemic hemodilution involves blood collection immediately before or even during surgery, with volume replacement and leading to a hematocrit below 0.32. Intraoperative blood salvage programs involve the collection of red cells during or after surgery from the operation site, and the reinfusion to the patient after a simple filtration or washing procedure. They are usually performed during surgery with direct overview by anesthesiologists and/or surgeons.
INFECTION PREVENTION AND DISPOSAL OF BIOHAZARDOUS WASTE

The RBTC should have in place programs designed to minimize risks to the health and safety of employees, donors, volunteers and patients. All personnel handling biohazardous specimens, should be given vaccination against Hepatitis B. Blood, blood components and its derivatives may contain infectious agents and, therefore, must be handled and disposed with precautions. All staff working in a blood center should be trained for infection prevention, disposal of biohazardous waste, treatment of spills and protocol for follow-up in the event of a needle stick or similar injury. Cleaning and decontamination of instruments and equipment should be performed on a regular basis as well as catering for occasional spillage. All efforts must be made to reduce generation of waste. All waste should be segregated at the site of generation into infectious and non-infectious categories. The infectious waste should be rendered noninfectious and disposed off as per governmental Biomedical Waste Rules (management and handling), 1998, Ministry of Forests and Environment, Government of India. This includes all units found positive for infectious diseases, records of which should be maintained with details of disposal.

QUALITY MANAGEMENT PROGRAM

An effective quality management program and good laboratory practices should be in place to ensure that the blood components and services meet standards acceptable to national guidelines. In addition, the RBTC may opt to fulfill the standards and requirements of national or international accreditation agencies such as the ISO. The quality management program includes a quality control/quality assurance program and documentation and quality evaluation tools.

The quality assurance program will ensure that policies and procedures are properly maintained and executed. It should be under the overall supervision of a QC officer who must be actively involved in determining methods and procedures, reviewing blood bank reports and quality control programs, and staff training and consultation. A blood bank quality manual and associated quality documentation should state the policies and operational procedures of the blood bank and should include details of the procedures for monitoring the validity of test results, for corrective action after detection of testing failures and departures from documented procedures, for auditing the blood bank, for reviewing the quality system and for dealing with complaints.

Quality Control Program

Quality control measures are instituted to ensure that reagents, equipment and methods function as expected and comply with the standards. They should include checking of supporting activities in addition to analytical and technical aspects. It requires the use of control materials appropriate to the test methods and material. The frequency of control testing will be such as to give assurance that the method is in control at the time of testing. Criteria must be established for the acceptance of control results as well as the action to be taken, including the withholding of test results, when the control results are unacceptable.

Documentation Systems

Documentation is a particularly important aspect of quality assurance and provides, among other things, the proof of compliance. These include among others, the quality manual, written standard operating procedures (SOPs); records and registers; work sheets, etc.

There should be detailed SOPs covering all GMP-compliant activities which will indicate actual practices and cover all phases of activity in all areas of the blood bank. Test methods best suited to fulfill the function of the RBTC should be selected; evaluated and authenticated based on national and international guidelines and manuals. Factors that should be considered for suitability should include sensitivity, specificity, speed, reliability, ease of use and economy. They should be clear and easy to follow so that they are usable by all involved in a process. It should include the source or reference for the procedure, the date the test was last reviewed, the calibration standards and controls required, instructions for handling specimens and reporting results, including appropriate reference ranges; and the step-by-step outline of the test method. SOPs should be reviewed periodically and at least annually.
Reporting of Test Results

Test reports should be furnished with minimum delay. These can be telephonic in an emergency but should be followed by a written report. No results of blood bank investigation or procedure should be given to the patient except with consent of the requesting medical practitioner. Disclosure of screening test results should be made to the donor in conformance with National standards and guidelines.

Recordkeeping

While SOPs provide the means of compliance with standards, records are the proof of compliance. Under GMP, it is not acceptable to simply state that a procedure has been carried out; there has to be documentary proof like interactive documents, e.g. work sheets to be filled in while carrying out a procedure. Records have to be correctly filled in, signed and dated. They must be retained for a sufficient length of time to allow confirmation of compliance with GMP, should this ever be called into question, professionally or legally.

All specimens should be fully identified and labeled and be available for a period of seven days after issue of blood. There should be a procedure established to ensure continued identification of specimens and blood products and records to ensure look-back procedures.

Minimum records and registers have to be maintained as per Drug Control rules and regulations and this includes details for blood donors and donations, screening, blood grouping, pre-transfusion tests and post-transfusion reactions work-up, blood components preparation, storage and issue; patient details, QC tests and checks, consumables used, blood discarded with reasons, work sheets of tests and so on.

Quality Tools

Quality tools consist of examination and assessment of all or part of a quality system with the specific purpose of improving it. They are External Quality Assessment Schemes (EQAS) and Internal Quality Audits. External quality assessment is concerned with examining and reporting the differences between different sites testing the same analyte (e.g. serum sample). Identical specimens are distributed to participants at regular intervals for assessment under the normal test conditions, results are returned to the organizer by the due date and reports are sent to all participants, highlighting poor performers. Each participant is given a code in order to preserve anonymity and encourage participation by all laboratories in a given region or country. These proficiency tests normally measure the adherence to, or deviation from, the norm of the rest of the participants, accuracy and precision of method, effects of changes in reagents and instruments, effects of changes in environment, effects of changes in personnel, and effects of changes in methods. The RBTC must participate in external proficiency testing quality assessment programs covering all test methods, which they perform and for which such programs are available.

Internal quality audits monitor compliance with a particular standard or guideline. Current schemes include good manufacturing practice (GMP) audits, certification audits to the International Standard Organization (ISO) 9001, accreditation audits and peer audits. For the purpose of conducting the audit, the quality audit (QA) unit will divide and subdivide the operations into systems, critical control points (areas that affect the safety and quality of blood if not performed correctly) and key elements (individual steps in each control point). The relationship of the different functions will be shown in an outline format referred to as a “function tree”.

Automation in Blood Banking

Automation of repetitive tasks can reduce human error and improve outcomes. The use of computerized record systems, automated analyzers, and robotic samplers has become commonplace in the developed world in the past twenty years. Computerization of blood bank data makes for easier storage and retrieval of data; better monitoring and audit of the blood bank functions and helps in blood use audits, look-back procedures and hemovigilance. Computerization should be done in a phased manner and start with the manual systems that work logically and rigorously. Introduction of computerization occurs next, when appropriate affordable equipment and software become available, using the manual systems as a model. It should always be remembered that a computerized system will not compensate for the shortcomings of an inadequate manual system.
Inventory Management

Inventory management involves optimizing the procurement, storage and use of equipment, spares, consumables and blood components in order to minimize the cost and effort, while maximizing the output of the blood center. It is an important part of good manufacturing practices (GMP).

Blood inventory management entails matching the supply with the demand for blood components, while at the same time minimizing outdating. The first-in-first-out principle works for all perishable goods, which includes blood as well as short-expiry consumables. The RBTC has to maintain its own inventory levels efficiently to cater to the blood needs of the hospitals and storage centers within its sphere of its responsibility, for regular as well as for emergency requirements. It has to take into account the distribution logistics of transport facilities and distance to the hospitals. The advantage of such a centralized system includes the ability to move blood components around between hospitals so that waste due to date-expiry is minimized.

One or more of the several blood inventory management techniques that are practiced through the world can be used. Whatever method is followed, it should specify blood-group-wise minimum inventory levels. The just-in-time supply principle has the advantage of fewer blood units wasted due to date-expiry but carries the risk of inventory shortage. It is far better to plan in advance, donor sessions and voluntary blood donation camps based on demand projections of the hospitals. However, predicting demand is not easy.

A simplistic method that is followed by many hospitals is the demand projections based on past average blood use per week or month. There are more sophisticated methods such as Cohen and Pierskalla’s model based on management techniques and mathematical tools. The National Blood Services in England and North Wales, for instance, have special demand-planning groups that regularly prepare short and medium term demand projections. Contingency planning for disaster management should include a trained emergency team; an automatic response system; identified donor groups and communication system.

Rational and optimal blood use as well as methods to reduce blood inventory should be put in place to optimize blood inventory management. The maximum surgical blood ordering schedules and standard blood orders are used by hospitals to reserve stocks for surgical requirements. The type and screen order is used for procedures not normally needing blood, e.g. averaging less than 0.5 units per patient per procedure, and helps to reduce blood units kept in reserve for a particular patient and reduces unnecessary crossmatches.

Blood use audits are a must and should be used to improve transfusion practice and thereby reduce blood inventory. In addition to the number and type of component used per procedure and per patient, the audit should also look at crossmatch : transfusion ratios, which should be less than two, unmet blood needs, wastage due to outdating, etc.

Inventory Control of Consumables

The inventory of consumables with limited shelf-life, e.g. blood bags, screening kits, reagent antisera, reagent red cells, other chemicals and emergency drugs, should be managed carefully to prevent date-expiry. Those with no expiry or long expiry, e.g. glassware, stationery, and linen, are easier to manage. A well-documented stock-control system should be in place under the supervision of the stores officer to ensure this.

FINANCIAL SUPPORT ISSUES FOR BLOOD BANKS

There are several types of blood banks and blood centers in India: hospital-based blood banks in government hospitals (public health system) and private hospitals, private, stand-alone, not-for-profit blood centers and private, commercial blood banks.

The blood banks in government hospitals are financed by the public health system. Some of the non-governmental blood centers get varying degrees of assistance by means of supply of certain consumables and equipment. But, for the most part, they have to manage their finances, which they do by recovery of minimal service charges to users. An RBTC will have to have a strong financial back up to cater to the setting up of the infrastructure requirements as well as to take care of the ongoing functions. Blood services are not meant to be profit centers, but they should aim to be financially viable. This is better if it is based on a cost-
recovery system because it is flexible with the scope for change to meet changing or increased budgetary requirements. A system based on funding, be it public or private, may not be dependable at all times. Service charges should be arrived at taking into account the costing of every activity involved in providing that product or service. This also results in sustainable cost-reduction efforts, both at the blood center as well as at the user level. The cost recovery should also take into account free or subsidized services that may be provided for the poorer sections of society. A professional financial planning and an annual budget and audits are a must. Plans should take into account not only regular expenses but also expenses due to repairs to building and equipment; inflation; increase in capacity to meet growing demands; accreditation, EQAS and look-back procedures, etc.

RESEARCH AND DEVELOPMENT

Ongoing research activities are essential as drivers for change, improvement and development. Epidemiological research to measure prevalence of infectious disease markers among different donor groups and identification of local risk factors can help an RBTC target safer donor groups, modify and tailor donor screening questionnaires and so on. Surveys about reasons why people do or do not donate blood, for regular and lapsed donations, etc. will have to be an ongoing exercise to spot donation trends and to tailor donor recruitment strategies. Research on blood transfusion use and abuse, prevalence of transfusion transmitted infections, etc. can form the basis for programs for clinicians to promote optimal and rational use of blood.

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SUGGESTED READING

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11. WHO Handbooks on Blood Banking and Transfusion Medicine.
A blood donor might need to see a physician after donating because of being deferred from blood donation due to hypertension, anemia or other reason or because of a positive infectious disease test found when donating or because of a complication experienced from blood donation. The purpose of this chapter is to provide information for clinicians so that they understand reasons for blood donor deferrals, the nature of the collection process, and some of the common and uncommon donor complications after phlebotomy.

The material is based on whole-blood collections in the United States and should be adapted to the local collection setting. Variations could be due to differences in whole-blood collection volumes, blood donor suitability criteria, and postdonation blood tests.

**BLOOD DONATIONS IN THE UNITED STATES**

Fourteen to 15 million volunteer whole-blood units are donated in the USA each year, and approximately 75 million whole-blood units are collected worldwide. There are essentially no paid whole-blood donors in the US, although it is legal to do so if one labels the blood as being from “paid” donors. Hospitals and the public do not desire such blood, as such blood has been shown to have a higher incidence of infectious disease when collected in metropolitan areas. Furthermore, whole-blood-derived components such as red cells and platelets cannot be sterilized. In contrast, the plasma collection industry routinely pays plasma donors US$15 to US$30 for the collection of plasma. Payment does encourage frequent donations and is considered to be the only way that the plasma industry could have sufficient plasma volume to meet its needs. Countries which depended solely on volunteer plasma were often unable to meet their needs, and imported plasma-derived products from the USA. This trend might be changing as more synthetic products enter the market and less plasma is needed. Plasma-derived products include albumin, immune serum globulin, factor VIII, and a host of other products.

Ninety-two percent of the whole-blood units collected in the US are collected by regional blood centers, and 8 percent are collected by hospitals. Approximately 95 percent of the whole-blood units collected are used for the community’s needs, while 4 percent are autologous units donated by patients for themselves prior to elective surgery, and 1 percent are directed donations. A directed donation is a whole-blood unit donated specifically for a relative or friend in need of blood. Regional blood centers generally collect from large groups to make the collection process cost-effective. Most of these collections are known as mobile collections because the blood center sends people and equipment to large groups such as large corporations, schools, houses of worship, government locations, and large social organizations. Another option is to collect from experienced blood donors at blood-center-owned- or leased sites; this permits selective recruitment of donors of specific blood groups.

Only 3 to 3.5 percent of the population donates blood each year in the USA. Approximately 80 percent of the donors are repeat donors, and they donate approximately 88 percent of the blood units. The average number of blood donations is approximately 1.6 per person per year. Females more often donate
blood than men. Fifty-seven percent of the donors presenting to donate in high schools in the Detroit metropolitan area (population 4.5 million) are females, and this trend continues through the age of 29 years. Other blood centers have also observed this trend. Males predominate after age 40 in large part because the hemoglobin acceptance standard of 12.5 g/dl favors males, who have higher hemoglobin concentrations than females, and because males have a higher retention rate due to fewer complications (23% vs. 46%) during or after whole-blood donation. Approximately 60 percent of the blood donors return within a year. The remaining blood comes from first-time donors (approximately 20% of all donors) and from donors who have not donated in a year or more.

A recent survey showed that every large metropolitan community is unable to uniformly meet its transfusion needs and must import blood. This is because blood usage is high in large cities, and blood centers in large cities have inadequate access to enough donors in the public to meet the need. In contrast, smaller communities have lower blood usage and can often collect more than they need and can export blood to other regions in need. In the USA, blood shortages tend to occur during the summer and at the end of December with extension into January because schools are closed, donors are away on vacation, and donors are less focused on blood donation. In addition, a blood center must have an adequate supply of each of eight RBC groups. These groups consist of all combinations of an ABO group (group O, A, B, and AB) and an Rh (Rhesus) type (positive or negative). Group O, present in 45 percent of the Caucasian population, is overtransfused because of trauma; is overtransfused when other non-O blood groups are absent from inventory; and is overtransfused for special attributes such as cytomegalovirus (CMV)-antibody negative blood, RBC antigen-negative blood, and neonatal usage. Group B, which is present in 11 percent of the Caucasian population, tends to be undercollected. Relative shortages are often limited to one or both of these two groups. In contrast, there is usually an excess of group A and AB red cells. In the Detroit area, approximately one-third to one-half of group AB red blood cell units are discarded at expiration rather than used.

Manual whole-blood collections accounts for at least 95 percent of current collections; but double-red-cell collections, using semiautomated apheresis machines, are increasing. This chapter will be limited to manual whole-blood collections.

**BLOOD DONOR SUITABILITY**

Blood donors must meet certain safety criteria to protect both the blood donor and the blood recipient. The Food and Drug Administration (FDA) and the American Association of Blood Banks (AABB) provide general blood donor suitability criteria for the blood collection industry, but each blood collection organization determines its own criteria for acceptance for the numerous diseases, conditions, and behaviors that they encounter. These criteria are often based on informed medical opinion.

Blood donors are eligible to donate whole blood once every eight weeks. Blood donors must be at least 17 years old in most states in the USA, weigh at least 50 kg (110 lb), and be in good health. The blood donor is given explanatory information about whole-blood donation upon arrival, and part of the information defines diseases, conditions, and behaviors that disqualify a donor. The donor can self-exclude at this point. Otherwise, acceptable health is determined with a health questionnaire, vital signs, inspection of the arm veins for evidence of drug use and a test for low hemoglobin. Blood donors can be accepted, permanently deferred, deferred for a specific time period, or deferred temporarily until the disqualifying disease, condition, or behavior is no longer an issue. Some deferrals are entered into an exclusion database in a computer and prevent the donor from being accepted on future visits.

**Blood Donor Suitability Criteria for Vital Signs and Inspection of the Arms**

The donor’s temperature cannot exceed 99.5°F or 37.5°C. The pulse must be between 50 and 100 beats per minute and regular. A pulse below 50 is acceptable, if the donor is a healthy athlete. A donor’s blood pressure cannot exceed 180/100 mm Hg. The antecubital fossa of both arms must not show any evidence of intravenous drug use, and the selected antecubital fossa should have no lesions, infection, or
scarring that could lead to a bacterial-contaminated unit.

**Blood Donor Suitability Criteria for Hemoglobin**

The FDA required minimum hemoglobin is 12.5 g/dl for both male and female donors. A capillary sample from the finger is used in all collection programs to measure hemoglobin or an equivalent 38 percent hematocrit. Hemoglobin concentration for 12.5 g/dl is often screened with a copper sulfate solution at a specific gravity of 1.053 because it is cheap, easy to use, and effective. Other potential methods are a microhematocrit determination or a photometric determination of hemoglobin concentration.

The current hemoglobin standard of 12.5 g/dl is below the normal range for Caucasian males (13.3–17.2 g/dl) and within the normal range for Caucasian females (11.6–15.7 g/dl) and African-American females (10.5–15.0 g/dl). This causes a much higher deferral rate for low hemoglobin in Caucasian females (15%) and African-American females (25–30%) than in males (1%) in the Detroit area. Such deferrals significantly decrease the overall donor pool. The deferral rates for low hemoglobin are reflected in the rates of deferral for all causes, which are 21 percent for females and 6 percent for males in the Detroit area. The latter difference, in turn, is a major cause of male predominance in the donor pool.

**Blood Donor Suitability Criteria for Diseases, Conditions, and Behaviors**

Two-thirds of the questions asked of donors in the American Red Cross (ARC) blood donation system relate to recipient safety criteria, and one-third relate to donor safety criteria. Prevention of recipient infections is a major goal. Criteria to ensure adequate blood component function are also important but are rarely an issue. The use of medications by the donor rarely causes deferral unless the underlying disease is an issue as with antibiotics for infection. On the donor safety side, determination of cardiac and pulmonary status and regular medical follow-up care for any medical conditions are some of the criteria that protect the donor. An in-depth review of donor suitability relative to numerous diseases, conditions, and behaviors is available. It should be noted that criteria change over time and vary among collection organizations, and they vary between countries.

**Deferral Rate**

The overall blood donor deferral rate was 13 percent in 2003 in Detroit. The five most common reasons for deferrals in Detroit in 2003 were low hemoglobin (8.1% of donors), elevated blood pressure (0.9%), travel to a malaria-endemic area (0.7%), elevated or irregular pulse (0.6%), and extended travel in a country with variant Creutzfeldt-Jakob disease (CJD) (0.4%).

**Confidential Unit Exclusion**

Approximately 0.22 percent of donors exclude their unit from transfusion through a process called confidential unit exclusion (CUE). The donor chooses one of two bar-coded “stickers”—one of the bar-codes means, “use my unit”, and the other bar-code means, “do not use my unit”. CUE provides an option for at-risk donors who are ineligible to donate but feel pressured to donate blood. By opting for the CUE step after donating, the donor can prevent the donated unit from being used as a blood transfusion. The CUE process, however, has marginal benefit; it is not required by the FDA, its use is optional, and half the donors who use it do so by mistake.

**Postdonation Tests**

Safety is further enhanced through postdonation testing for HIV-1 and -2, hepatitis B virus, hepatitis C virus, human T-lymphotropic virus types I and II, and West Nile virus. Approximately 1 percent of units are discarded due to a positive screening test and most of these are false positives (e.g. for anti-HIV-1 and -2, anti-HTLV types I and II, syphilis, anti-HCV, and HBsAg) or are positive but are for nonspecific tests (e.g. anti-HBc). Recently, the College of American Pathologists and the AABB have produced standards that require blood centers and transfusion services to test for bacteria in platelet components. Culture-based methods are feasible in the blood centers; but simpler and more rapid techniques, such as pH or glucose measurement, are more practical in transfusion services.
**BLOOD DONATION PROCESS**

Donor identification is confirmed by the phlebotomist after the donor has satisfied the medical history, blood pressure, pulse, temperature and hemoglobin requirements. An antecubital vein is selected and prepared with two iodine-based antiseptic solutions to reduce skin bacteria. A large 16-gauge needle is used to ensure adequate blood flow, thereby minimizing the occurrence of blood clots in the collection bag. The blood and the anticoagulant in the bag are also mixed several times during the phlebotomy to prevent clot formation. A typical venipuncture or collection failure rate is approximately 2 to 4 percent. To reduce the risk of hypovolemia and vasovagal reactions, an AABB standard requires that the blood collection volume should not exceed 10.5 ml/kg of donor weight. In the American Red Cross, the current collection volume consists of 481 ml in the collection bag, 33 ml for blood tests, and 11 ml in the tubing, for a total of 525 ml. This limitation on collection volume reduces the risk of hypotensive reactions and ensures compliance for the lowest acceptable allogeneic blood donor weight, which is 50 kg (110 lb). In some Asian countries, the collection volume is much lower. For instance, in Japan, there are two whole-blood collection volumes, 200 ml and 400 ml. The average blood collection time for 500 ml is approximately 7 minutes, with a range of 4 to 20 minutes. A full unit that is collected in less than 4 minutes usually signifies an arterial puncture. The collection bag typically has one or two additional plastic bags attached via integral plastic tubing. This permits further processing via centrifugation in a closed system (not open to the environment) into packed red blood cells, platelets, and fresh frozen plasma (FFP).

**BLOOD DONATION ADVERSE EVENTS**

Approximately one-third of whole-blood donors have an adverse physical event during or after whole-blood donation of 500 ml. In most cases, it is a minor event; but if the donor feels it is significant, the donor might visit a physician. The incidence of seeking outside medical care for an adverse event is at least 1 in 3,400 blood donations or 0.033 percent. Therefore, physicians should be familiar with the recognition, treatment and prognosis of blood donation complications. Prevention of adverse events, where possible, is important to minimize the number of blood donor injuries, but adverse events are an inevitable part of whole-blood donation. This chapter will emphasize the advances in knowledge over the last several years.

**Incidence of Blood Donor Injuries and Reactions**

Blood donor syncopal reactions and injuries were evaluated in 1,000 blood donors based on observation and a postdonation interview three weeks later. Table 4.1 shows the incidence of common and uncommon reactions and injuries. The most common adverse event, an ecchymosis or bruise at the phlebotomy site, occurs in 23 percent of donors and is more common in females (31%) than males (13%). Hematoma, defined as a collection of blood under the skin, is less common, occurring in 1.7 percent of the donors. The other common adverse events are phlebotomy site pain or tenderness (10%), fatigue (8%), and a vasovagal hypotensive reaction without syncope (7.0%), while uncommon events include nerve irritation or injury (0.9%), syncope (0.1–0.3%), and arterial puncture (0.01%).

**Vasovagal Reaction**

A vasovagal reaction refers to the presence of any of the following symptoms and signs during or shortly after a blood donation: apprehension, pallor, dizziness, weakness, diaphoresis, nausea, hypotension, and

<table>
<thead>
<tr>
<th>Table 4.1: Incidence of whole-blood donor complications</th>
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<tr>
<td>Arm injuries</td>
</tr>
<tr>
<td>Bruise</td>
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<tr>
<td>Pain</td>
</tr>
<tr>
<td>Hematoma</td>
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<tr>
<td>Nerve irritation</td>
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<td>Local allergy</td>
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<td>Arterial puncture</td>
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<td>Thrombophlebitis</td>
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<td>Local infection</td>
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<td>Systemic</td>
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<tr>
<td>Fatigue</td>
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<tr>
<td>Vasovagal reaction</td>
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<tr>
<td>Syncope</td>
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<tr>
<td>Nausea/vomiting</td>
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<tr>
<td>Systemic—MI, stroke, etc.</td>
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<tr>
<td>TOTAL (Donors affected)</td>
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</table>
The overwhelming majority of donors will not have a reaction, even in high-risk groups. The incidence of a vasovagal reaction is 2 to 3 percent based on observation alone, and 7 percent based on observation and a follow-up interview three weeks later. A vasovagal reaction is more common in young, low-weight, and first-time blood donors, and there can be synergism among factors. There is also a psychological component because reactions can occur prior to phlebotomy or in some donors upon observing a donor with a reaction (epidemic fainting).

The major contributing factors to the vasovagal reaction rate of 1,000 interviewed blood donors were found to be age and weight using a stepwise regression analysis. First-time donor status also contributed but to a lesser degree. Race was also a contributor to vasovagal reactions. Caucasian high-school students had a higher vasovagal reaction rate than African-American high-school students (8.2% vs. 1.3%) in a study of 1,076 Caucasians and 226 African-Americans. In a larger, more definitive study of 8,500 first-time donors, Caucasians had a 2.4-fold higher vasovagal reaction rate than African-Americans (8.3% vs. 3.4%); and the same was true when the high-school subgroups were compared (14.5% vs. 6.3%). These data provide strong evidence that Caucasians have a higher vasovagal reaction rate than African-American blood donors. Females also have a higher vasovagal reaction rate than males, irrespective of weight, when sufficient numbers of high-risk donors are studied. In 8,135 first-time, 17-year-old Caucasian students, females had a higher vasovagal reaction rate than males (16.2% vs. 7.1%); and there was a sex-specific difference at every 10 lb interval between 120 and 200 lb. No sex-specific differences were found when low-risk donors or small numbers of high-risk donors were evaluated.

There are approximately 25 nonsyncopal vasovagal reactions for every syncopal reaction. Most nonsyncopal vasovagal reactions occur on the blood-donation bed toward the end of the phlebotomy or immediately after the phlebotomy. Syncopal reactions, in contrast, occur more often when the donor stands after donating. In 10 to 15 percent of the cases, the syncopal reaction occurs after the donor has left the blood donation site, and most of these syncopal reactions occur within the first hour. These donors are more likely than donors with vasovagal reactions at the collection site to be sent to the hospital emergency room and more likely to have inappropriate resuscitation efforts started. Fourteen percent of the donors who have syncope sustain an injury upon falling, and it is usually to the head. These injuries tend to be minor; but, in rare instances, the blood donor can sustain a fracture or closed-head injury. Donors spontaneously recover from a vasovagal reaction; but, if the recovery is prolonged or syncope is recurring, the donor can be sent to an emergency room for observation. The incidence of health care visits for vasovagal reactions is approximately 1 in 9,300 donations, and approximately one-third of these visits relate to injuries (unpublished ARC, 2003 data). The author is not aware of any blood donor deaths related to syncopal vasovagal reactions.

A vasovagal reaction is also an issue for blood donor retention because a nonsyncopal vasovagal reaction decreases the blood donor return rate by 30 to 56 percent after a one-year follow-up, and syncope decreases the donor return rate even further, to 53 to 76 percent. Three approaches for prevention of vasovagal reactions have been suggested. One approach is to use an automated apheresis machine to collect a smaller-volume red cell unit from an at-risk donor and return additional fluid to the donor. Automation of double-red-cell collections results in fewer vasovagal reactions than whole-blood collections; but cost, availability of blood processing intruments, and logistics remain challenges for single RBC-unit collections. A second approach is to have high-risk donors drink 16 fluid ounces (473 ml) of water approximately 30 minutes prior to phlebotomy. Water drinking is able to ameliorate severe orthostatic hypotension in elderly patients and also decreases vasovagal reactions in healthy subjects subjected to tilt-table tests. A side issue is that predonation drinking of fluid can cause a slight decrease in hemoglobin concentration (–0.1 to –0.7 g/dl) in approximately 70 percent of subjects, so the hemoglobin screening test in females should ideally be done prior to drinking the water.
Reduction of the collection volume would be expected to reduce vasovagal reactions significantly, but relatively similar costs for different collection volumes and more allogeneic transfusions per patient make this an unlikely approach. Other methods such as predonation drinking of coffee, muscle tension, greater attention to the donor, distraction, and having a well-organized blood drive might also help but are difficult to precisely define or manage or require informed consent.

Nerve Injury or Irritation

Occasional nerve injuries are unavoidable after phlebotomy because nerve branches cannot be palpated or seen. In one study, 40 percent of the nerve injuries occurred after a straightforward, uncomplicated phlebotomy. Horowitz added additional evidence that these nerve injuries are unavoidable because he found significant variation in nerve branch anatomy in autopsy studies of seven pairs of arms. Nerve branches were found below the antecubital veins, but they were also found above antecubital veins and intertwined with them. A nerve injury complaint is uncommon; but out of seven million donations, approximately 1 in 4,400 donors complained of symptoms consistent with a nerve injury (ARC, 2003 data). Approximately one in 22,000 blood donors visit a health care provider for evaluation of the injury (ARC, 2003 data). Common complaints are sensory changes away from the venipuncture site—often in the forearm, wrist, hand or shoulder—or radiating pain. Approximately 25 percent of the nerve injuries are associated with hematomas. Nerve injuries are almost always transient events. Forty percent disappear within a few days; another 30 percent, within a month; 23 percent, within 1 to 3 months; and 7 percent, within 3 to 9 months. Treatment is symptomatic, but referral to a neurologist specializing in peripheral nerve injuries should be considered for severe cases. In some cases, there is residual numbness but the arm is fully functional. In very rare cases, the donor develops complex regional pain and a permanent disability.

Iron Depletion in the Donor

The average female and male iron stores are 300 mg and 1,000 mg, respectively. A whole-blood donation removes approximately 200 to 250 mg of iron. While males can tolerate this loss, females have less iron reserve. Approximately 11 percent of the menstruating female population is iron deficient, with higher rates in African-American females (19%) and Hispanic females (22%). Seventy-five percent of women have dietary iron intakes that are below the United States Department of Agriculture’s Recommended Dietary Allowance (RDA) of 15 mg/day, and 20 percent have intakes below 50 percent of the RDA. Just 20 to 25 percent of menstruating women take an iron supplement. Thus, many women are susceptible to iron depletion or iron deficiency anemia after blood
donation. This may lead to a concern for possible blood loss if the donor sees a physician with complaints of fatigue or if hemoglobin screening is done. Iron depletion also affects cognitive function, and studies have shown that iron deficient, nonanemic adolescent and adult females do less well on memory tasks.49,50 Mental function is decreased further when iron depletion progresses to iron deficiency anemia.50 A prudent preventative approach is to provide short-term iron supplements for menstruating females after successful whole-blood donation.

Postdonation Hospitalization or Death

Boynton and Taylor described 10 deaths that occurred in temporal proximity to 7 million whole-blood donations during the World War II.21 It was determined that the incidence was lower than expected for the general population. It is believed that most deaths related to the blood donation are coincidental and not caused by the donation. Sazama described 12 donation-related deaths reported to the FDA between 1976 and 1985.51 Of the 12 donors, 8 were plasma donors from the plasma industry and one was a white-blood-cell donor. Of the three whole-blood donors, two donors had a myocardial infarction and one had a pheochromocytoma. The author has not observed a death in 2.4 million whole-blood donations.

Popovsky et al described hospitalizations from 4.1 million whole-blood donations.52 The incidence was approximately 1 in 200,000 for allogeneic whole-blood donation and 1 in 17,000 for autologous blood donation. The most common adverse events causing the hospitalization were vasovagal reaction (73 percent), angina (12%), and a variety of arm injuries (15%).

CONCLUSIONS

Approximately 83 percent of donors who attempt to donate succeed; but 13 percent are rejected because of blood donor suitability issues; 1 percent have a positive test, which is often nonspecific or false-positive; and 2 to 4 percent of the phlebotomies are unsuccessful. The most common donor adverse events are bruise (23%), sore arm (10%), fatigue (8%), and vasovagal reaction (7%). One in 3,400 donors or 0.033 percent report seeking outside medical care. Hospitalization is rare; the most common reason for a hospitalization is a vasovagal reaction, but hospitalizations also occur for chest pain and arm injuries. Seriously injured are possible but are very rare. Overwhelmingly, blood donors do well and feel satisfied with the blood donation experience.

REFERENCES

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48. USDA, ARS. Data tables: Results from USDA’s 1994-96 Continuing Survey of Food Intakes by Individuals and 1994-96 Diet and Health Knowledge Survey. Riverdale,


Leukocytes and cytokines produced by leukocytes that are contained in blood components can cause several adverse outcomes of transfusion (Table 5.1). Febrile reactions may occur from antibodies to donor leukocyte antigens or from cytokines released from blood component leukocytes during storage. The leukocytes in red cell and platelet components express HLA antigens and can stimulate the formation of HLA antibodies in the recipient. This can lead to refractoriness to platelet transfusion. The development of HLA antibodies in transfusion recipients can threaten the success of future stem cell or organ transplantation, if needed. Engraftment of transfused donor T lymphocytes in recipients who have received a hematopoietic stem cell transplant can cause fatal graft-versus-host disease (GVHD). Several viruses are carried by leukocytes, including cytomegalovirus (CMV), and removing leukocytes from blood components may prevent their transmission by transfusion. Controversial issues surrounding leukocytes in blood components include their role in inducing immune suppression in recipients that seem to be associated with increased incidence of postoperative infection and tumor recurrence. Removal of white blood cells from blood components may possibly help reduce bacterial content in contaminated components and reduce the risk of reperfusion injury following periods of cardiac ischemia and coronary artery bypass surgery. Because of these real and potential benefits, this chapter addresses the clinical effectiveness of leukocyte reduction of blood components and recommendations for its use.

**LEUKOCYTE REMOVAL**

**Mechanisms, Timing, and Potential Adverse Effects**

The use of leukocyte-reduction filters is the most effective and common method to remove leukocytes from blood components. Other methods such as differential centrifugation, sedimentation, washing, and freezing/thawing are used less often. White blood cells (WBCs) are removed by filters because of their larger size compared with red blood cells (RBCs) and by adherence to certain types of fibers. Filters in common use remove between 3 and 5 log₁₀ (99.9–99.999%) of WBCs in RBC and platelet components and reduce the WBC content to less than 5 × 10⁶ per unit, the threshold for many of the adverse effects of transfused leukocytes. Function of red cells and platelets appear not to be affected by filtration, and without the presence of leukocytes during storage,
cellular integrity and post-transfusion recovery are improved by modest, yet reproducible amounts. On the other hand, filtration may result in the loss of up to 15 to 25 percent of red cells and platelets, thereby reducing the administered cell dose and potentially incurring the costs and risks of additional transfusion.

Blood component filtration can be performed in the blood collecting facility during the collection of apheresis platelets, or shortly after whole-blood donation and preparation of blood components (pre-storage). Post-storage leukocyte filtration may occur in the hospital laboratory or at the bedside prior to transfusion. Pre-storage filtration appears to have several advantages over bedside filtration, including:

1. better quality control of the physical variables that affect filter performance (ambient temperature, duration of filtration, etc.);
2. a reduction of the cytokine production during storage and a lower incidence of febrile reactions and alloimmunization; and possibly
3. diminished immunomodulation that may result from the transfusion of WBC membrane fragments.

Two types of reactions have been related to the effects of leukocyte-reduction filtration. Several hypotensive reactions have been reported in patients taking angiotensin-converting enzyme (ACE) inhibitors following the administration of leukocyte-reduced blood components prepared with the use of negatively, not positively, charged filters at the bedside. These reactions are similar to those that have been observed during therapeutic apheresis and during hemodialysis when patients have been taking ACE inhibitors. The etiology is thought to be activation of the kallikrein system with the generation of bradykinin triggered by the exposure of plasma to negatively charged surfaces. Since ACE is responsible for the breakdown of bradykinin, the bradykinin levels can remain elevated in patients taking an ACE inhibitor and transient hypotension can occur.

The other reaction type is a novel one not previously associated with transfusion and possibly caused by a certain lot of filters. In 1997 and 1998, the Centers for Disease Control and Prevention received reports of a transfusion-related "red eye" syndrome consisting of severe conjunctival redness, ocular pain, periorbital edema, arthralgias, and headache. These reactions occurred only after the transfusion of leukocyte-filtered components prepared at the bedside with a particular filter, but the underlying mechanism remains to be determined.

Whether leukoreduction filtration is applied to all cellular blood transfusions ("universal leukoreduction") or just to some, quality control techniques are required to ensure that blood components meet the standards for leukocyte reduction and do so consistently. In the United States, the American Association of Blood Banks' (AABB) Standard 5.7.4.1 requires that leukocyte-reduced blood and components shall be prepared by a method known to reduce the leukocyte number to less than $5 \times 10^6$. Validation and quality control must demonstrate that at least 95 percent of units sampled meet this criterion.

Validation and quality control must demonstrate that at least 95 percent of the units sampled meet this criterion. European standards require fewer than $1 \times 10^6$ WBCs in leukocyte reduced blood components. When counting leukocytes, proper attention must be given to obtaining an adequate sample with adequate stripping of the tubing. Since the white cell counts are too low to be enumerated by the usual hematology cell counters, residual leukocytes are counted by the Nageotte Chamber Method or by flow cytometry or another similarly sensitive technique.

Leukocyte reduction failures can be seen during filtration of blood from donors with sickle cell trait, which causes filter blockage and failure to remove WBC in units that do not freely flow through the filter. Filtration failure is likely related to sickle hemoglobin polymerization as unsuccessful leukocyte reduction and clogging of the filter are associated with lower pO$_2$ and higher osmolality anticoagulants.
In addition to filtration, leukocyte reduction of apheresis platelets may be achieved by special automated apheresis collection techniques. Certain apheresis collection instruments reliably yield a therapeutic dose of platelets accompanied by fewer than $1 \times 10^6$ leukocytes per component. In fact, most of these instruments produce components with much fewer leukocytes than those required for the component to be considered leukocyte-reduced. Both apheresis-derived and routinely filtered platelet components have similar efficacy in removing leukocyte subset populations. The very low levels of residual leukocytes of any type make it unlikely that these two approaches to leukocyte reduction will be clinically discernible in their effects.

FEVER AND RIGORS IN RECIPIENTS AND LEUKOREDUCTION

Fever is a common consequence of the transfusion of components containing allogeneic leukocytes and it occurs in approximately 1 percent of RBC\textsuperscript{32} and up to 30 percent of platelet\textsuperscript{32-34} transfusions. A febrile, nonhemolytic transfusion reaction (FNHTR) has been defined as a rise in patient temperature of 1ºC, with or without chills and rigors, once other causes of fever (e.g. infection or hemolysis) have been ruled out.\textsuperscript{35} Some hospitals use a rise in temperature of 1 or 2 oC resulting in a temperature of at least 38ºC as a trigger to stop the transfusion. However, studies have also shown these symptoms may occur in the absence of fever in some patients receiving leukocyte-reduced blood components.\textsuperscript{36} In addition to the patient discomfort associated with these reactions, the laboratory and clinical evaluations of these reactions increase the cost and delay the benefits of transfusion.\textsuperscript{37} Fortunately, many, but not all, FNHTRs may be prevented through the use of leukocyte-reduced blood components.

Several mechanisms may explain the role of donor leukocytes in the pathogenesis of fever in transfusion recipients.\textsuperscript{2} In all three models, the final common pathway for the production of fever is the release of inflammatory cytokines [e.g. interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)] from leukocytes, and increased plasma cytokine concentrations have been observed in patients experiencing FNHTRs.\textsuperscript{38} These inflammatory mediators stimulate prostaglandin synthesis in the hypothalamus that results in fever.\textsuperscript{39} In the first model of an FNHTR, recipient antibodies are directed against HLA or non-HLA antigens on donor leukocytes. Fever results when antibody binds to the transfused leukocytes, causing the release of pyrogens such as IL-1 from donor leukocytes. Antibodies directed against HLA, granulocyte-specific, and platelet-specific antigens have been detected in several patients experiencing FNHTRs,\textsuperscript{40-43} but more severe reactions have been associated with granulocyte\textsuperscript{42} antibodies. While plausible, this model fails to explain FNHTRs that occur in patients lacking antileukocyte antibodies or in patients receiving leukocyte-reduced platelets.\textsuperscript{37,44-46} In the second model, formation of antigen-antibody complexes causes complement activation, with C5a stimulating the release of inflammatory cytokines from recipient monocytes.\textsuperscript{47-49} Recent evidence now supports a third model for the occurrence of FNHTRs following transfusion: the accumulation of cytokines in blood components through active synthesis during storage.\textsuperscript{34,50-55,63-71}

Several clinical observations suggest that cytokines (e.g. IL-1, IL-6, and TNF) produced during storage of cellular blood components may be a major cause of FNHTRs.\textsuperscript{34,50-55} First, FNHTRs are more common following the transfusion of platelets (which are stored at 20–24ºC) than after the transfusion of RBCs, even though RBCs contain more leukocytes.\textsuperscript{33,34,44,72} The increased incidence of FNHTRs seen with platelet transfusion may correlate with increased cytokine production at room temperature compared with RBCs stored at 4ºC.\textsuperscript{66} Second, the incidence of FNHTRs increases with the age of the component, again suggesting production and accumulation during storage.\textsuperscript{34,50-52,54,56-58} Third, FNHTRs occur in patients who have not been pregnant or previously transfused and who, therefore, should not have an antileukocyte alloantibody to trigger the reaction.\textsuperscript{33,50} Fourth, leukocyte reduction does not prevent all reactions. Fifth, Heddle and colleagues\textsuperscript{50} observed a higher frequency of FNHTRs following transfusion of the plasma rather than of the cellular component of whole-blood-derived platelet concentrates (PCs). Also, plasma removal is effective in lowering the incidence of FNHTRs to platelets.\textsuperscript{58-61} In fact, only about 10 percent of FNHTRs are associated with HLA antibodies in the transfusion recipient.\textsuperscript{50,62} Taken
together, these observations are consistent with the production of soluble, donor-derived, inflammatory cytokines during blood component storage that are not removed by post-storage leukocyte-reduction filtration. As expected, pre-storage leukocyte reduction is effective in preventing cytokine accumulation in blood components during storage, whereas post-storage filtration is not.

The efficacy of leukocyte-reduced RBCs in the prevention of FNHTRs is well documented. Removal of 75 to 90 percent of the leukocytes from a unit of RBCs to below $5 \times 10^8$ prevents most FNHTRs in multitransfused patients with a history of recurrent FNHTRs. In such patients, the incidence of these reactions diminished from 10.3 to 1.3 percent following microaggregate filtration, an older leukocyte-reduction technique with about 90 percent removal efficiency. In multiply transfused thalassemia patients (who experience a high incidence of FNHTRs), post-storage leukocyte-reduction filtration decreased the incidence of these reactions from 13 to 0.5 percent. In patients with hematologic malignancies, the exclusive use of post-storage, leukocyte-reduced RBCs resulted in an FNHTR rate of 2.15 percent. Pre-storage filtration appears to be more effective than post-storage filtration in preventing FNHTRs but does not eliminate these reactions entirely. Several retrospective studies have examined the efficacy of universal pre-storage leukocyte reduction (ULR) for the prevention of FNHTRs. All but one of these studies observed a decrease in FNHTRs following ULR. In the prospective VATS study, there was no difference in FNHTRs in HIV-infected patients, although the baseline rate of fever associated with transfusion was high. In the only randomized controlled trial of pre-storage leukocyte reduction which enrolled all patients without an established indication for receiving leukocyte-reduced blood components, those receiving leukocyte-reduced components had a numerically but not statistically ($p = 0.06$) lower rate of febrile reactions.

While both pre- and post-storage leukocyte reduction are effective in reducing FNHTRs associated with RBC transfusion, prevention of cytokine accumulation by pre-storage filtration is most effective in preventing febrile reactions to platelet transfusion. Early reports of bedside filtration found a decrease in FNHTRs in leukocyte-reduced platelets, but the small number of patients and transfusions that were evaluated limits their conclusions. Mangano and colleagues found a lower incidence of FNHTRs with post-storage leukocyte-reduced platelet units; the rate of febrile reactions in this group of patients dropped from 27 to 17 percent for those receiving whole-blood-derived platelets and from 14 to 7 percent for those receiving plateletpheresis units. In contrast, Goodnough et al found that bedside filtration caused no difference in the reaction rates to platelet transfusion. Similarly, a prospective, clinical study of patients with hematologic malignancies showed no difference in the incidence of FNHTRs with the use of post-storage leukocyte-reduced components. The clinical effectiveness of pre-storage leukocyte reduction in diminishing FNHTRs to platelet transfusion exceeds that of post-storage leukocyte reduction; in a study of hematology/oncology patients with a history of at least two prior febrile reactions, those who subsequently received post-storage leukocyte-reduced plateletpheresis units had a 3.5-fold higher reaction rate (4.50%) than those who received leukocyte-reduced plateletpheresis units prior to storage (1.28%). The role of pre-storage leukocyte reduction in decreasing the rates of FNHTRs is supported by two of three retrospective and one prospective study showing a lower incidence of such reactions following the introduction of ULR.

In summary, removal of leukocytes is effective in preventing the majority of FNHTRs. Pre-storage filtration is more effective than post-storage filtration, especially in platelet components stored at room temperature where metabolically active WBCs generate proinflammatory, fever-inducing cytokines.

**HLA ANTIBODIES AND REFRACTORINESS TO PLATELET TRANSFUSION**

Alloimmunization to Class I HLA antigens on platelets can cause of refractoriness to platelet transfusion, which occurs in 30 to 70 percent of multi-transfused patients. A poor 1-hour post-transfusion corrected count increment (CCI) suggests that alloimmunization is present. A poor CCI may also be due to other clinical factors, including fever, infection, splenomegaly, disseminated intravascular coagulation, veno-oclusive disease, amphotericin and vancomycin therapy, occurrence of a transfusion reaction or ABO
Alloimmunization occurs most commonly to HLA Class I alloantigens, although platelet-specific antibodies may also develop, albeit at much lower frequency. Transfusion strategies to overcome the refractoriness to platelet transfusion associated with the presence of HLA and/or platelet-specific alloantibodies include the use of HLA-matched, phenotypically negative or crossmatched platelets. In patients, who already have broadly reactive HLA antibodies, these platelet selection techniques may have limited efficacy, and life-threatening thrombocytopenia may persist in spite of multiple platelet transfusions. The frequency with which refractoriness causes hemorrhagic morbidity or mortality remains to be determined. Therefore, the prevention of alloimmunization in patients who are likely to be multiply transfused is preferable to the management of refractoriness once it develops.

Methods to prevent alloimmunization to HLA and platelet-specific alloantigens include the use of leukocyte-reduced or ultraviolet (UV)-irradiated components. Platelets express only Class I antigens and are a poor stimulus of primary alloimmunization. Exposure to HLA Class II antigens present on leukocytes in transfused blood components or during pregnancy appears to be necessary for the development of antibodies to HLA Class I antigens. Further, the development of HLA antibodies requires Class I and II antigens to be present on the same cell, as third-party leukocytes fail to produce alloimmunization. UV irradiation of platelet components reduces the recipient's humoral immune response by interfering with presentation of donor antigens to helper T lymphocytes. Although not approved for routine use in the United States, UV irradiation prevents alloimmunization with no apparent effect on platelet function or in vivo survival.

Numerous studies have evaluated the clinical efficacy of leukocyte reduction in the prevention of alloimmunization and platelet refractoriness (Table 5.2). Most of these reports found a lower incidence of alloimmunization and refractoriness in patients transfused with leukocyte-reduced components. The development of alloantibodies appears to be dose-dependent, as studies with more effective leukocyte removal are associated with less frequent alloimmunization. The threshold number of leukocytes per component to reduce alloantibody formation appears to be approximately $1 \times 10^6$. In fact, two of the studies that failed to show an effect of leukocyte reduction involved transfused components with WBC exceeding this level.

Recent evidence suggests that leukocyte reduction prevents primary alloimmunization, but is less efficacious in avoiding HLA antibody development and refractoriness in patients previously sensitized by pregnancy or previous transfusion. The development of alloantibodies and refractoriness is 2- to 10-fold higher and occurs sooner in women with a history of pregnancy than in immunologically naive patients. The Trial to Reduce Alloimmunization to Platelets (TRAP) study found that the use of leukocyte-reduced components did not lower the incidence of refractoriness in patients with a history of pregnancy compared with control patients; however, their use halved the rate of alloantibody formation in previously pregnant women. In contrast, Seftel et al and Sintnicolaas et al did not find a benefit of leukocyte reduction in preventing alloimmunization or platelet refractoriness in patients with a history of previous pregnancy. On the other hand, leukocyte reduction was effective in patients previously sensitized by transfusion. These results are supported by observations that pre-storage leukocyte reduction virtually eliminates primary sensitization but not a secondary response. The transfusion of platelets (which express Class I, but not Class II, HLA antigens) may stimulate an anamnestic response leading to lymphocytotoxic antibody formation. Thus, while further study is necessary to determine if patients who have been pregnant or transfused previously will benefit from leukocyte-reduced blood components to prevent platelet alloimmunization, rates of refractoriness appear to be similar.

Leukocyte reduction with current technology does not prevent all primary alloimmunization from occurring as a result of platelet transfusions. Alloimmunization may result from the transfusion of WBC fragments that accumulate during platelet, but not RBC, storage and may pass through post-storage leukocyte-reduction filters. In an animal model, pre-storage leukocyte reduction is associated with less alloimmunization and refractoriness than post-storage leukocyte reduction. In addition, the removal of plasma and leukocytes is more effective than leukocyte...
reduction alone.\textsuperscript{137} It remains to be seen if pre-storage filtration will be more effective in preventing alloimmunization in the clinical setting. A greater reduction in platelet refractoriness was seen in the Canadian\textsuperscript{133} experience using pre-storage filtration compared to the TRAP study\textsuperscript{77} which employed post-storage filtration. However, it is difficult to compare these studies as they differ in experimental design and the rates of alloimmunization and refractoriness in the control groups were quite different (Table 5.2). Thus, it remains to be seen if pre-storage filtration will be more effective in preventing alloimmunization.

Leukocyte reduction prevents primary alloimmunization, but is it cost effective? Added expenses associated with the transfusion of leukocyte-reduced components include the cost of the filter (pre-storage or post-storage) and the extra labor required to perform the filtration. Also, since it is not possible to predict which patients will form alloantibodies, leukocyte-reduced components must be provided to all patients at risk of becoming refractory. On the other hand, the added costs associated with transfusing alloimmunized patients include the need for additional transfusions and use of HLA or cross-matched platelet products. A 1997 report calculated the mean added cost of hemotherapeutic support for marrow transplantation patients who become refractory may have been as much as $15,000.\textsuperscript{138} Cost savings through the use of leukocyte-reduced components has been reported in patients with leukemia and lymphoma.\textsuperscript{123,139,140} The savings were realized through decreased platelet use, decreased use

### Table 5.2: Leukocyte reduction and alloimmunization

<table>
<thead>
<tr>
<th>Study (ref)</th>
<th>Patients (n)</th>
<th>HLA alloimmunity (%)</th>
<th>Platelet refractoriness (%)</th>
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<td>530</td>
<td>45%</td>
<td>17-18%</td>
</tr>
<tr>
<td>Settel, 2004</td>
<td>617</td>
<td>61/315 (19)</td>
<td>21/302 (7)</td>
</tr>
</tbody>
</table>

LR: Leukocyte-reduced; PLT: Platelet; NA: Not available; ND: Not determined

* p>0.05
† p not determined, otherwise p<0.05
‡ Not randomized
§ Reference group received mostly leukoreduced components
Primary alloimmunization
¶ Secondary alloimmunization
# Pre-storage leukocyte reduction
of HLA or crossmatched platelets, and shortened hospital stays. The use of fewer HLA-matched platelets alone did not offset the added cost of filtration. Additional savings may result from a more rapid hematopoietic recovery and lower infection rate following hematopoietic stem cell transplantation in patients with AML who receive leukocyte-reduced blood components. These studies are encouraging and suggest that leukocyte reduction is cost effective for select patient populations. With additional information regarding the clinical benefits and cost savings related to avoiding alloimmunization and refractoriness, clinicians may be better able to identify those patients most likely to benefit from the use of leukocyte-reduced blood components.

PREVENTING CYTOMEGALOVIRUS TRANSMISSION BY LEUKOREDUCTION

Cytomegalovirus (CMV) is a leukocyte-associated virus that may be transmitted by blood components. Infection in immunocompromised patients is associated with considerable morbidity and mortality (e.g. pneumonitis, gastroenteritis, retinitis) and is best prevented. In fact, given the devastating effects of CMV infection following hematopoietic stem cell transplantation, many centers now employ “preemptive” treatment with antiviral agents like ganciclovir in patients demonstrating molecular evidence of acute CMV infection by PCR. Persons at risk for the adverse sequelae of CMV infection are listed in Table 5.3 and include low birth weight neonates, CMV-seronegative pregnant women, HIV-

<table>
<thead>
<tr>
<th>Category*</th>
<th>Clinical circumstance</th>
<th>CMV-Seronegative Blood (Unmodified)</th>
<th>Leukocyte-Reduced (LR) Blood (CMV-Unscreened)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CMV+ patient</td>
<td>Not indicated</td>
<td>Not indicated</td>
</tr>
<tr>
<td></td>
<td>CMV- patient</td>
<td>Not indicated</td>
<td>Not indicated</td>
</tr>
<tr>
<td>II</td>
<td>CMV+ patient</td>
<td>Not indicated</td>
<td>Use of LR blood to prevent viral reactivation</td>
</tr>
<tr>
<td></td>
<td>CMV-patient</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
</tr>
<tr>
<td>III</td>
<td>CMV+ recipient</td>
<td>Not indicated</td>
<td>Not indicated</td>
</tr>
<tr>
<td></td>
<td>CMV- recipient of CMV+ organ donor</td>
<td>Not indicated</td>
<td>Not indicated</td>
</tr>
<tr>
<td></td>
<td>CMV- recipient of CMV- organ donor</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
</tr>
<tr>
<td>IV</td>
<td>CMV+ recipient</td>
<td>Not indicated</td>
<td>Use of LR blood to prevent viral reactivation</td>
</tr>
<tr>
<td></td>
<td>CMV- recipient of CMV+ donor</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
</tr>
<tr>
<td></td>
<td>CMV- recipient of CMV- donor</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
</tr>
<tr>
<td>V</td>
<td>CMV+ recipient</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
<td>LR blood may be slightly preferred to CMV-seronegative blood (passive CMV immunoglobulin)</td>
</tr>
<tr>
<td></td>
<td>CMV- recipient</td>
<td>Either CMV-seronegative blood or LR blood is indicated</td>
<td></td>
</tr>
</tbody>
</table>

* Category I patients: General hospital patients and general surgery patients (including cardiac surgery). Patients receiving chemotherapy that is not intended to produce severe neutropenia (adjuvant therapy for breast cancer, treatment of CLL, etc.) Patients receiving corticosteroids (patients with immune thrombocytopenic purpura, collagen vascular diseases, etc.) Full-term infants

Category II patients: Patients receiving chemotherapy that is intended to produce severe neutropenia (leukemia, lymphoma, etc.). Pregnant patients, HIV-infected individuals

Category III patients: Solid organ allograft patients who do not require massive transfusion support

Category IV patients: Patients receiving allogeneic and autologous hematopoietic progenitor cell transplants

Category V patients: Low birth weight (< 1200 g) premature infants
infected individuals, severely neutropenic chemotherapy patients, recipients of seronegative solid organ allografts and hematopoietic stem cell transplant patients regardless of the CMV status of donor. Transfusion-associated CMV (TA-CMV) may be minimized through the use of seronegative blood components. For example, such components have reduced the infection rate in CMV-seronegative marrow transplantation patients from 1 to 4 percent compared with an incidence of 23 to 37 percent seen with the transfusion of CMV-unscreened blood components.\textsuperscript{144-147}

Given the residual risk of CMV transmission with seronegative components, several groups have examined whether CMV nucleic acid testing of blood donors might reduce this risk. Roback and colleagues\textsuperscript{148} found only 2 of 1,000 (0.2%) whole-blood donors were positive for CMV DNA, and both were positive by antibody screening. In addition, Greenlee et al\textsuperscript{149} found no CMV DNA in 203 donors, 110 of whom were CMV seropositive. On the other hand, cell-free viremia occurs in recently infected donors who have not yet developed anti-CMV antibodies, which supports the clinical evidence that CMV antibody screening may not be 100 percent effective in preventing CMV transmission by transfusion.\textsuperscript{150} Thus, the potential of CMV NAT screening to detect seronegative, infectious units remains promising, but with current technology for CMV nucleic acid testing, screening for blood donors seems premature.\textsuperscript{151}

The use of leukocyte-reduced blood components as an alternative to CMV-seronegative products is important for several reasons. First, the seroprevalence of CMV in the blood donor population is high (50–90%); which limits the number of seronegative units available for transfusion.\textsuperscript{143} Second, the added cost of donor testing for CMV would be eliminated for patients receiving leukocyte-reduced blood components for other clinical indications. Third, leukocyte reduction might prevent the transmission of CMV by seronegative units that test falsely negative. Fourth, \textit{in vitro} spiking studies demonstrate leukocyte-reduction filters achieve a 2 to 3 log removal of CMV DNA from WB and buffy coat platelets.\textsuperscript{152-154} Fifth, currently available apheresis technology also achieves a similar removal of CMV DNA from apheresis platelets.\textsuperscript{155} Numerous studies have examined the efficacy of leukocyte reduction in the prevention of TA-CMV\textsuperscript{156-169} (Table 5.4). Although many studies were limited in design, taken together they provide strong support for the prevention of TA-CMV by leukocyte-reduction filtration in adult patients. The evidence for the efficacy of leukocyte reduction in neonates is less clear.\textsuperscript{170} Of the four studies, two\textsuperscript{165, 167} were conducted in areas endemic for CMV, yet other modes of CMV transmission, e.g. via cervical secretions or breast milk, were not examined.\textsuperscript{171} The other two studies\textsuperscript{158, 163} had a pooled odds ratio of 0.19 (95% CI, 0.01–3.4), indicating a clinical effect that was not statistically significant.\textsuperscript{170} Given these study limitations, and the adverse clinical outcomes of CMV infection, the use of CMV safe blood for all or, at least, susceptible neonates is recommended. The randomized controlled trial of 502 marrow transplantation patients by Bowden and colleagues\textsuperscript{147} demonstrated the efficacy of leukocyte reduction in the prevention of TA-CMV infection. However, the conclusion that leukocyte-filtered blood components are equally as effective as CMV-seronegative blood components remains controversial.\textsuperscript{145, 172} In the primary analysis of the study data, the rates of CMV infection and disease occurring in the period of 21 to 100 days after transplant were equivalent. Infections observed before day 21 were not considered to be transfusion related as they were either acquired prior to entry into the study or reflected false-negative patient CMV screening test results upon study entry. In contrast, a secondary analysis of the data from the date of transplant (days 0–100) showed equivalent rates of CMV \textit{infection} but a higher incidence of CMV \textit{disease} in the leukoreduced group, although the number of affected patients was small (n = 6 filtered, n = 0 seronegative, p = 0.03). In spite of the controversy over the data interpretation, this study clearly demonstrates that the use of leukocyte-reduced blood components decreases TA-CMV to levels seen with seronegative blood components.

Recently, Nichols and colleagues\textsuperscript{173} questioned the equivalence of CMV-seronegative and leukocyte-reduced red blood cells (RBCs). They compared two historical periods where CMV-seronegative components were provided to provide CMV safety (Period 1; 5/96-11/96) or leukoreduced units were
provided (Period 2; 12/96-2/2000). Retained samples were tested post-transfusion to determine the CMV serostatus of all units. While more apheresis products were provided in period 2, multivariate analysis demonstrated increased risk of CMV antigenemia in recipients was associated with leukoreduced RBCs but not platelets. This retrospective study comparing two historical time periods has several limitations. First, there was a higher number of transfusions per patient in the group receiving leukoreduced components. Second, only a small number of transfused components were leukocyte reduced in either period (0.7–1.5% of RBCs: 1.2–15.8% of platelets). Third, more apheresis platelets were used in period 2; all of these components may not have met the AABB standard of 5 × 10^6 residual WBC per component. Since a small fraction of components were used for WBC QC, the rate of filter failure is not known. Nevertheless, this study raises two important questions: (i) Are leukocyte-reduced and CMV-seronegative blood red cells equivalent? (ii) Is there an added benefit to patients of providing CMV-seronegative components in the era of universal leukocyte reduction?

A recent Canadian consensus conference addressed these unresolved questions. The consensus panel reviewed the literature and summarized our current dilemma when comparing leukocyte-reduced and CMV-seronegative blood components for prevention of CMV transmission:

- Both are effective
- Neither is perfect
- Cannot conclude one is better than the other
- Any benefit of CMV seronegative and leukocyte reduction together is not known

Not surprisingly, the group was divided on providing CMV-seronegative together with leukocyte-

<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>Number of patients</th>
<th>Diagnosis</th>
<th>Post-Transfusion CMV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-LR</td>
</tr>
<tr>
<td>Verdonck, 1987113</td>
<td>29</td>
<td>Bone marrow transplant</td>
<td>NA</td>
</tr>
<tr>
<td>Murphy, 1988114</td>
<td>20</td>
<td>Acute leukemia</td>
<td>2/9 (22)</td>
</tr>
<tr>
<td>Gilbert, 1989115</td>
<td>72</td>
<td>Neonates</td>
<td>9/42 (21)</td>
</tr>
<tr>
<td>De Graan-Hentzen, 1989116</td>
<td>145</td>
<td>Leukemia, lymphoma</td>
<td>10/86 (12)</td>
</tr>
<tr>
<td>Bowden, 1989117</td>
<td>17</td>
<td>Bone marrow transplant</td>
<td>NA</td>
</tr>
<tr>
<td>DeWitte, 1990118</td>
<td>28</td>
<td>Bone marrow transplant</td>
<td>NA</td>
</tr>
<tr>
<td>Bowden, 1991119</td>
<td>65</td>
<td>Bone marrow transplant</td>
<td>7/30 (23)</td>
</tr>
<tr>
<td>Eisenfeld, 1992120</td>
<td>48</td>
<td>Neonates</td>
<td>NA</td>
</tr>
<tr>
<td>van Prooijen, 1994121</td>
<td>48</td>
<td>Bone marrow transplant</td>
<td>ND</td>
</tr>
<tr>
<td>Xu, 1995122</td>
<td>27</td>
<td>Neonates</td>
<td>9/12</td>
</tr>
<tr>
<td>Bowden, 1995112</td>
<td>502</td>
<td>Bone marrow transplant</td>
<td>ND</td>
</tr>
<tr>
<td>Preiksatis, 1997123</td>
<td>76</td>
<td>Children with malignancy</td>
<td>0/32</td>
</tr>
<tr>
<td>Ohto, 1999167</td>
<td>52</td>
<td>Neonates (CMV endemic)</td>
<td>1/19 (5)</td>
</tr>
<tr>
<td>Navrios, 2002168</td>
<td>36</td>
<td>Bone marrow transplant</td>
<td>ND</td>
</tr>
<tr>
<td>Rhonge, 2002169</td>
<td>93</td>
<td>Bone marrow transplant</td>
<td>ND</td>
</tr>
<tr>
<td>Nichols, 2003173</td>
<td>807</td>
<td>Stem cell transplant</td>
<td>NA</td>
</tr>
</tbody>
</table>

LR = leukoreduced

- Equivalent to background seroconversion in infants (>90% of mothers are seropositive)
- Infection at day 21–100 post-BMT, p = 1.0
- Disease at day 21–100 post-BMT, p = 0.25
- Infection at day 0–100 post-BMT, p = 0.5
- Disease at day 0–100 post-BMT, p = 0.03*
- Period 2: Unfiltered apheresis products collected LR also provided. See text for full details
- Period 1: Only CMV seronegative and/or filtered products provided
reduced blood components (1/10, no; 2/10, pregnant women only; 7/10, yes – provide both). Although the additional benefit accruing with the use of both techniques simultaneously could only be projected, it appeared to be small although attractive.

Although the AABB guideline (Table 5.3) supports the equivalent efficacy of leukocyte-reduced and CMV-seronegative blood components in preventing TA-CMV,\textsuperscript{143} the US Food and Drug Administration (FDA) Blood Products Advisory Committee failed to support the equivalent safety for these two approaches.\textsuperscript{175} In a meeting with the AABB, the FDA representatives indicated that before the agency could recommend the use of leukocyte-reduced products, each filter manufacturer must submit data proving equal efficacy. In the meantime, “the state of the art right now is CMV-screened products,” according to Jay Epstein, MD.\textsuperscript{176} Apart from these product claim issues, many transfusion services are using leukocyte-reduced components instead of, or in conjunction with, CMV seronegativity to reduce the risk of CMV transmission by blood components, a practice with which the author agrees.

Several patient populations are most likely to benefit from CMV-reduced-risk blood components (Table 5.3). In contrast to the guidelines, some clinicians provide CMV-reduced-risk components only to neonates weighing less than 1,200 g who were born to CMV-seronegative mothers rather than to all such low-birth-weight neonates. Similarly, some clinicians transfuse CMV-safe components only to CMV-seronegative hematopoietic progenitor cell transplant recipients who receive CMV-seronegative transplants rather than to all CMV-seronegative recipients. Such practices are acceptable. As a practical point, because all hematopoietic progenitor cell transplant recipients are likely to be receiving leukocyte-reduced blood components, they are all likely to be transfused with CMV-reduced-risk components.

Infection with a Second-Strain of CMV

Restriction endonuclease analysis has been used to demonstrate that transplantation of seropositive solid organs into seropositive recipients may result in recipient infection with a second strain of CMV.\textsuperscript{177-180} Although second-strain infections occur, they are associated with less symptomatic disease than seen with primary infection.\textsuperscript{183-182} By analogy with solid organ transplantation, there is the possibility of transmission of second-strain infection by the transfusion of CMV-unscreened blood components, but this has not been reported. Adler et al\textsuperscript{183,184} found equivalent rates of CMV infection in seropositive cardiac surgery patients who received either CMV-unscreened (7/48) or seronegative (5/46) blood. Similarly, Winston and colleagues\textsuperscript{185} found no second-strain infections in a small study of marrow transplant patients and that symptomatic disease represented reactivation of latent infection. The multicenter Viral Activation Transfusion Study (VATS) did not find a significant risk of second-strain infection in HIV-infected patients. Only two of twenty-five evaluable patients had CMV genotype shifts that could not be correlated with the genotype of the transfused unit.\textsuperscript{186} Given the lack of evidence of transmission by transfusion and the lower morbidity associated with second-strain infections in solid organ transplantation, the use of CMV-reduced-risk blood components to prevent recipient infection with a different strain of CMV is not recommended.\textsuperscript{143}

Reactivation of Latent CMV Infection

The reactivation of latent CMV infection occurs in immunocompromised patients and may also be associated with blood transfusion. In animal models, the culture of allogeneic cells or the transfusion of allogeneic blood stimulated the reactivation of CMV infection.\textsuperscript{187-189} The effect of a transfusion may depend on immune status, as one study demonstrated reactivation only in animals that had received total body irradiation.\textsuperscript{187} In cardiac surgery patients, Adler and McVoy\textsuperscript{184} found increased CMV titers following the transfusion of CMV-seronegative and CMV-unscreened units. CMV reactivation correlated with the number of units transfused and was not seen in nontransfused patients. Two small studies of CMV-seropositive heart transplant patients have demonstrated that the use of leukocyte-reduced blood components is associated with less CMV disease (0/17, 0%) than the use of non-leukocyte-reduced components (14/36, 43%).\textsuperscript{190,191} While these studies were encouraging, the multicenter Viral Activation Transfusion Study (VATS) found no changes in CMV viral load in either CMV-negative or CMV-positive patients.\textsuperscript{186,192} Similarly, no viral reactivation was seen with other viruses including HBV, HCV, HTLV I/II,
Therefore, the use of leukocyte-reduced blood products to prevent viral reactivation is not recommended.

**TRANSFUSION ASSOCIATED IMMUNE SUPPRESSION AND LEUKOREDUCTION**

The immunosuppressive effect of allogeneic transfusion on renal allograft survival was observed by Opelz 30 years ago, and an additive effect is still seen with current immunosuppressive medications.\(^{194,195}\) In addition, allogeneic transfusion reduces the recurrence rates of spontaneous abortion\(^{196}\) and inflammatory bowel disease,\(^{197,198}\) and it may improve engraftment and survival in hematopoietic stem cell transplant patients.\(^{122}\) On the other hand, potential deleterious effects of transfusion-induced immunosuppression include reactivation of viral infections and increases in postoperative bacterial infection and tumor recurrence. The immunomodulation seen with allogeneic transfusion likely results from a decrease in cell-mediated immunity.\(^{199}\) The observed effects of allogeneic transfusion include a shift from a Th1 to a Th2 immune response, as well as decreases in natural killer cell activity, the CD4/CD8 ratio, and lymphocyte blastogenesis.

The role of leukocytes in mediating the immunomodulatory effects of allogeneic transfusion remains controversial despite numerous animal, observational, and randomized clinical trials. Blajchman and colleagues\(^{137,200,201}\) demonstrated that mice and rabbits receiving allogeneic blood either before or after inoculation with fibrosarcoma cells have increased pulmonary metastases compared with animals receiving syngeneic blood. This tumor growth-promoting effect could be transferred to naive animals by the infusion of splenic T cells derived from animals that had received allogeneic transfusion previously. The effect on tumor growth was eliminated in animals receiving pre-storage but not post-storage leukocyte-reduced blood. This observation suggests that cytokines may mediate the immunosuppressive effect, which is supported by the fact that increased tumor growth was seen with stored syngeneic blood but not with fresh syngeneic blood. Further, the intra-peritoneal injection of spleen cells enclosed in diffusion chambers also was associated with enhanced tumor growth. Thus, the results from animal models suggest that the transfusion of leukocytes present in allogeneic blood enhances tumor growth through soluble inflammatory mediators.

Several recent studies suggest transfusion of soluble receptors and/or their ligands may cause immune downregulation. Fas/Fas ligand inhibits cytotoxic T-cell activity and induces apoptosis. The concentration of soluble Fas ligand is higher in stored cellular blood products compared to fresh components or those that have been pre-storage leukocyte reduced.\(^{202,203}\) In a murine model, allogeneic blood transfusion is associated with increased expression of Fas and Fas ligand on CD4+ and CD8+ spleen cells.\(^{204}\) In addition, less Fas/Fas ligand expression and apoptosis is seen with transfusion of pre-storage leukocyte-reduced blood.

The results of studies that have examined the effect of allogeneic transfusion on malignancy and postoperative infection rates in patients are less clear. While approximately two-thirds of the nearly 100 observational studies found that allogeneic transfusion is associated with increased cancer recurrence, a large number of studies have not observed this effect.\(^{205}\) These studies are limited by their design. They used statistical techniques to control for the effects of confounding variables on recurrence rates.\(^{206,207}\) Several meta-analyses of the literature have been performed in an attempt to reach a conclusion from these studies.\(^{206-208}\) Chung et al\(^{208}\) found a cumulative odds ratio for cancer recurrence of 1.8 [95% confidence interval (CI) = 1.30-2.51]. Vamvakas\(^{206}\) found a smaller effect; the relative risk for colorectal cancer recurrence was 1.60 (95% CI = 1.27–2.02). However, the deleterious effect of transfusion on tumor recurrence was eliminated when only prospective studies, which showed a relative risk of 1.18 (95% CI = 0.93–1.51), were included in the analysis.

Three randomized clinical trials have examined the effect of allogeneic transfusion on cancer recurrence.\(^{209-211}\) While Heiss et al\(^{209}\) found a lower rate of cancer recurrence in patients receiving autologous rather than allogeneic blood, the study by Busch and colleagues\(^{210}\) did not. In a secondary analysis, transfused patients, regardless of whether they received allogeneic or autologous blood, had a worse prognosis than patients who did not require transfusion. These studies assume that autologous transfusion has no immunosuppressive effect, which
may not be the case if immune downregulation occurs via soluble mediators as discussed above. Therefore, studies comparing the risks of autologous and allogeneic blood might underestimate the effect of allogeneic transfusion on tumor recurrence. In a multicenter clinical study, Houbiers and colleagues compared the transfusion of leukocyte-reduced and buffy coat-depleted RBCs on colorectal cancer recurrence and no significant difference was observed between the two groups. In a meta-analysis of these three randomized clinical trials, there was no effect of allogeneic transfusion on tumor recurrence (odds ratio, 1.04; 95% CI, 0.81–1.35). Nevertheless, a clinical trial examining the effect of leukocyte reduction on cancer recurrence has not been performed in the United States, where some components have higher leukocyte concentrations because buffy coats are routinely not removed from red cell units. Thus, the immunosuppressive effect of allogeneic transfusion on cancer recurrence and whether the use of leukocyte-reduced components can reduce this risk remain a matter of debate.

The majority of more than 30 observational studies have demonstrated that perioperative transfusion is associated with increased postoperative infections. In a retrospective review, Vamvakas and Carven found increased rates of postoperative pneumonia in cardiac bypass patients receiving non-leukocyte-reduced blood and the risk increased with the length of storage of the blood components. In contrast, two studies compared the rates of postoperative infection in two historical time periods, before and after the introduction of universal leukocyte reduction, and found no difference in infection rates.

Ten randomized clinical trials have examined the effect of transfusion on the rate of postoperative infections (Table 5.5). Two of the clinical trials compared the postoperative infection rate in colorectal cancer surgery patients receiving either allogeneic or autologous transfusion. While Heiss et al. found a significantly decreased postoperative infection rate in patients transfused with autologous compared with buffy-coat-depleted blood (12% vs 27%), Busch et al. found similar rates of infection (27% vs 25%). While three of eight prospective studies that examined the effect of leukocyte-reduced blood compared with allogeneic blood found a beneficial effect on postoperative infection, the remaining five did not. Comparisons of these studies is difficult given differences in study design; all but one were performed in Europe utilizing buffy-coat-removed RBCs, and the overall number of patients is small. In a meta-analysis of six of these clinical trials, the relative risk associated with allogeneic transfusion was 0.94 (95% CI = 0.85 – 1.04, p > 0.05). In a mathematical model, Vamvakas and Blajchman concluded a study involving 3,000 patients would be necessary to detect a 10 percent difference in the risk of postoperative infection in patients transfused with leukocyte-reduced blood. For perspective, the clinically accepted practice of using allogeneic leukocytes to prevent recurrent spontaneous abortions is effective in only 10 percent of patients.

### Table 5.5: Randomized clinical trials of allogeneic transfusion and postoperative infection

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Diagnosis</th>
<th>Postoperative infection rate</th>
<th>p</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Auto</td>
<td>Allo</td>
<td>LR</td>
</tr>
<tr>
<td>Heiss217</td>
<td>120</td>
<td>Colorectal cancer</td>
<td>12</td>
<td>27</td>
<td>N.D.</td>
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<tr>
<td>Busch210</td>
<td>475</td>
<td>Colorectal cancer</td>
<td>27</td>
<td>25</td>
<td>N.D.</td>
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<tr>
<td>Jensen218</td>
<td>104</td>
<td>Colorectal cancer</td>
<td>N.D.</td>
<td>23</td>
<td>2</td>
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<tr>
<td>Jensen219</td>
<td>260</td>
<td>Colorectal surgery</td>
<td>N.D.</td>
<td>12</td>
<td>0</td>
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<tr>
<td>van der Watering220</td>
<td>909</td>
<td>Cardiac surgery</td>
<td>N.D.</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>Houbiers211</td>
<td>334</td>
<td>Colorectal cancer</td>
<td>N.D.</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>Tartter221</td>
<td>59</td>
<td>GI surgery</td>
<td>N.D.</td>
<td>44</td>
<td>16</td>
</tr>
<tr>
<td>Wallis224</td>
<td>509</td>
<td>Cardiac surgery</td>
<td>N.D.</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Titstage223</td>
<td>112</td>
<td>Colorectal surgery</td>
<td>N.D.</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>Houbiers222</td>
<td>446</td>
<td>Colorectal surgery</td>
<td>N.D.</td>
<td>36</td>
<td>42</td>
</tr>
</tbody>
</table>

N.S. = not significant
N.D. = not determined
the effect of leukocyte reduction of blood components prepared by the platelet-rich plasma (PRP) method should be examined before conclusions can be made about transfusion and use of these components. To address this question, Dzik et al.230 conducted a large prospective study of all patients lacking a standard indication for receiving leukocyte-reduced components prepared by the PRP method. In this study, there was no observed difference in mortality or the incidence of postoperative infection as assessed by antibiotic usage. Thus, demonstration of the potential beneficial effect of leukocyte reduction on postoperative infection rates remains elusive.

Although the debate over the potential immunosuppressive effect of perioperative allogeneic transfusion continues, even a small effect would have a profound impact on morbidity, mortality, and the costs associated with transfusion in surgical patients. Blumberg226 estimated that 2,150 deaths per year in the United States would be related to the immunosuppressive effect of transfusion if it caused a 10 percent increase in tumor recurrence and postoperative infection. If a beneficial effect were attributable to leukoreduction, the potential cost savings might be substantial (Table 5.6). Jensen et al.227 found savings in total hospital charges for colorectal cancer patients receiving either leukocyte-reduced whole blood ($7,867) or no transfusion ($7,037) compared with those receiving transfusion with allogeneic blood ($12,347). Subsequent studies215,220,221,223,227-232 had mixed results related to cost and length of stay; only four of seven,221,227-229 demonstrated a significant cost reduction whereas three of nine227,228,232 showed a significant reduction in the length of stay associated with the use of leukocyte-reduced blood components (Table 5.6). Dzik and colleagues230 conducted the only large-scale randomized controlled trial not limited to certain patient subgroups to evaluate the possible cost-benefit of universal leukocyte reduction. There was no benefit of leukocyte reduction on cost or length of stay in all patients studied or in subgroups of patients undergoing cardiac surgery or colorectal surgery. Thus, while cost savings may occur in selected groups of patients, the economic benefit of universal leukocyte reduction remains to be demonstrated.

Finally, allogeneic transfusion could have an adverse clinical effect in HIV-positive patients through immunosuppression or virus reactivation. Transfusion of patients infected with HIV has been associated with a more rapid progression to acquired immunodeficiency syndrome (AIDS) and higher mortality.233-236 Although these epidemiologic findings suggest that the need for transfusion may be related to more severe underlying disease, several findings suggest it may be due to immunomodulation or virus reactivation. For example, the expression of HIV by infected T cells appears to require immunologic stimulation of these cells.237-239 Busch and colleagues240 found that allogeneic leukocytes stimulated a dose-dependent

<table>
<thead>
<tr>
<th>Study</th>
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* Postoperative stay
N.D. = Not determined
N.S. = Not significant
³ Patients without an established indication for leukocyte-reduced blood products
** Historical comparison
rise in HIV expression in infected cells in culture. Mudido et al\textsuperscript{241} reported an increase in HIV p24 antigen and HIV RNA following transfusion in nine patients. In a small prospective study, however, Groopman\textsuperscript{242} demonstrated that leukocyte reduction did not eliminate HIV reactivation by transfusion. Furthermore, the VATS study found no viral reactivation associated with non-leukocyte-reduced allogeneic blood transfusion for HIV, CMV, HBV, HCV, HTLV I/II, or HHV-8.\textsuperscript{192,193} Thus, transfusion of allogeneic leukocytes does not appear to induce clinically significant viral reactivation.

**LEUKOREDUCTION AND BACTERIAL CONTAMINATION OF BLOOD COMPONENTS**

The transfusion of blood components contaminated with bacteria may cause septic transfusion reactions. These reactions may be characterized by a combination of fever, rigors, hypotension, tachycardia, hemoglobinuria, and disseminated intravascular coagulation. As the risks of viral transmission by transfusion have been dramatically reduced, attention has focused on bacterial contamination as a significant cause of transfusion-associated morbidity and mortality. The incidence of septic transfusion reactions may be as high as 1 in 350 pooled, random-donor platelet concentrates,\textsuperscript{243,244} 1 in 2,000 to 3,000 for single units of platelets derived from whole-blood units\textsuperscript{245-247} and apheresis units, and 1 in 31,000 RBC transfusions.\textsuperscript{243} During the period of 1986 to 1991, bacterial contamination was implicated in 16 percent (29/182) of all transfusion-related fatalities reported to the FDA.\textsuperscript{250} The most common organism contaminating platelets is *Staphylococcus epidermidis*. *Yersinia enterocolitica*, which can grow at refrigerated temperatures, is the most common pathogen associated with septic reactions to transfusion of RBC components.\textsuperscript{252}

Bacterial contamination of blood components occurs mainly at the time of venipuncture as a result of inadequate skin preparation or asymptomatic donor bacteremia. Contaminating organisms from the skin appear to be found primarily in the first 5 to 10 ml of blood collected and may be associated with a skin plug.\textsuperscript{253}

In an effort to decrease the incidence of septic transfusion reactions, the AABB implemented a new standard (# 5.1.5.1), effective March 1, 2004 which requires blood banks and transfusion services to employ methods to limit and detect bacterial contamination of platelet products.\textsuperscript{254} The College of American Pathologists had previously (December, 2002) included a similar requirement in its accreditation checklist (TRM.44955). Methods to limit contamination include proper skin preparation and diversion of an initial aliquot of blood for infectious disease testing. Diversion of the first 10 to 15 ml of collected whole blood has been shown to reduce the incidence of bacterial contamination of whole-blood units by 40 to 70 percent.\textsuperscript{255,256} Unfortunately, implementation of sample diversion by blood centers in 2004 was associated with increased hemolysis in sample tubes, and production by several manufacturers has been temporarily suspended. The most sensitive method to detect contamination of platelet components currently is bacterial culture near the time of collection. Less-sensitive methods to detect bacteria need to be performed near the time of transfusion after bacteria have grown to detectable levels. Post-storage methods include gram/acridine orange staining and measurement of unit pH and/or glucose. Platelets swirling is only acceptable in emergency situations. These methods are discussed in the AABB Association Bulletin # 03-10.\textsuperscript{257}

Several *in vitro* studies have demonstrated that leukocyte-reduction filters are capable of removing bacteria from inoculated RBCs or whole blood.\textsuperscript{252,258-265} Units spiked with *Y. enterocolitica* or coagulase-negative *Staphylococcus* (CNS) and leukocyte reduced by filtration several hours after collection had less bacterial contamination than unfiltered components. On the other hand, leukocyte-reduction filtration is less efficacious in removing bacteria from platelets. At low and high inoculum concentrations of CNS after overnight storage, filtration reduces, but does not eliminate bacterial contamination of buffy coat-derived platelet units.\textsuperscript{265} Similarly, filtration is partially effective in minimizing bacterial contamination in platelet-rich plasma-derived platelet units stored for 10 days.\textsuperscript{264} Wenz and colleagues\textsuperscript{266} demonstrated a delay in bacterial *growth* in leukocyte-reduced platelets, but no difference in contamination rate was seen after day 1 of postfiltration storage. Similarly, molecular analysis of bacterial RNA showed equivalent growth rates in platelets containing *S. epidermidis*.\textsuperscript{267}
Leukocyte-reduction filters may remove bacteria by two mechanisms. First, filtration may remove bacteria directly, given that *Staphylococcus xylosus* can be extracted from components that have been leukocyte reduced previously. Filters have removed bacteria from saline but not from 5 percent albumin, which apparently alters the interaction of organisms with the filter surface. The direct removal of bacteria from FFP but not from heat-inactivated plasma suggests that bacterial adherence to the filter surface may be complement dependent. Second, leukocyte filters also may remove phagocytized bacteria present within WBCs. These bacteria might have been released back into the blood if the leukocytes underwent cell death and fragmentation before the bacteria were killed. The optimal time for filtration is probably between 2 and 12 hours after collection, a period that would permit phagocytosis and removal of leukocytes prior to the later release of viable organisms, a mechanism that has been cited to explain bacterial growth in a unit previously found to be sterile. Thus, the removal of small amounts of bacteria may be an added benefit of pre-storage leukocyte reduction. However, implementation of methods to limit and detect bacterial contamination and emerging pathogen inactivation technologies may be more effective strategies to reduce transfusion of bacterially contaminated blood components than leukocyte reduction. Leukoreduction by adsorption filtration cannot be relied upon to generate a sterile unit, particularly when the filtration is applied after storage.

**LEUKOCYTE REDUCTION AND GRAFT-VERSUS-HOST DISEASE**

In hematopoietic progenitor cell transplant patients, the engraftment of donor T lymphocytes may cause graft-versus-host disease (GVHD). Similarly, the transfusion of cellular blood components containing viable donor lymphocytes may cause transfusion-associated graft-vs-host disease (TA-GVHD), which is more severe because TA-GVHD is characterized by pancytopenia caused by the engrafted cells attacking the recipient’s marrow, a feature absent from post-transplantation GVHD. Most cases are fatal. Fortunately, TA-GVHD is quite rare in immunocompetent transfusion recipients as donor lymphocytes are usually cleared rapidly by recipient cytotoxic (CD8+) T cells. The majority of donor cells (99.9%) are removed by day 2 following transfusion; however, a small number of lymphocytes may persist in the circulation for a month or more.

Immunocompromised individuals at risk for TA-GVHD include hematopoietic stem cell transplant recipients, patients with hematologic malignancies or solid tumors, patients with T-cell immunodeficiencies, individuals receiving granulocyte transfusions, and fetuses receiving intrauterine transfusions. In addition, numerous cases of TA-GVHD have been reported in neonates. In a review of the Japanese literature, the majority of neonatal TA-GVHD cases (24/27) were associated with the transfusion of fresh blood. These observations indicate that the transfused lymphocytes must be viable to achieve engraftment and cause tissue damage.

Recent reports suggest that TA-GVHD disease may occur in patients treated with nonmyeloablative regimens containing the purine analogues fludaribine and cladribine. Of the ten reported fatal cases, the diagnoses included: B-cell chronic lymphocytic leukemia (CLL), B-cell non-Hodgkin’s lymphoma (NHL), acute myeloid leukemia (AML) and systemic lupus erythematosus (SLE). In another interesting case, a patient with CLL treated with an autologous peripheral blood stem cell (PBSC) transplant developed fludaribine-associated TA-GVHD which was successfully treated with re-transplantation of back-up PBSCs. Fludaribine causes a T-cell lymphopenia that may persist for up to a year following the treatment period. Given the morbidity and mortality associated with TA-GVHD, the author feels the use of irradiated products should be considered in patients treated with fludaribine.

TA-GVHD may also occur in immunocompetent transfusion recipients who are heterozygous for an HLA haplotype for which the donor is homozygous. Such a situation and thus the risk of TA-GVHD is higher in directed donations from relatives and may be increased as much as 21-fold in donations from parents to children. The effect of donations from close relatives is noted again in the Japanese data where, in one series, 22 of 27 TA-GVHD cases occurred following the transfusion of blood from relatives. Similarly, in a recent report by Aoun and colleagues from Lebanon, nine of ten cases TA-GVHD occurred following transfusion of fresh, non-leukocyte reduced,
Leukoreduction of Blood Components

non-irradiated blood. The necessary combination of HLA types can occur in situations where the donor and recipient are unrelated, of course. Although this chance occurrence is more likely in a society in which there is less genetic diversity, the chance of it occurring in a transfusion pair in the United States is surprisingly high: approximately 1/22,000. The lower than expected incidence of TA-GVHD may be due to underreporting; all ten cases in one center’s experience were seen in immunocompetent individuals.

The prevention of TA-GVHD is accomplished by gamma irradiation of cellular blood components, which inactivates T lymphocytes by DNA. Frozen plasma products have not caused TA-GVHD and are not routinely irradiated, although they may contain a significant number of viable lymphocytes. However, several cases of transfusion-associated GVHD have been associated with fresh plasma. Irradiation must ensure a midplane dose of at least 25 Gy and a minimum 15 Gy to the remainder of the blood component. In fact, TA-GVHD has been reported in children with AML and ALL who received blood components that had been irradiated at 15 to 20 Gy. The threshold number of leukocytes capable of causing TA-GVHD appears to be \(10^4/\text{kg}\). Currently available leukocyte-reduction filters are capable of approaching this level of leukocyte reduction, which suggests that they may decrease TA-GVHD. Dzik and Jones were able to demonstrate a dose-dependent exponential decrease in the mixed lymphocyte reaction model of GVHD using leukocyte-reduced blood. However, a case of TA-GVHD has been reported in a patient receiving components prepared exclusively by leukocyte-reduction filtration (albeit with the use of a less-effective filter than is currently available). Therefore, leukocyte reduction must not be relied upon as a substitute for gamma irradiation of blood components to prevent TA-GVHD. Emerging pathogen inactivation technologies inactivate T lymphocytes and may reduce the risk of TA-GVHD, but these technologies have not been approved in the United States.

Transfusion of immunocompetent T lymphocytes may have beneficial effects in hematopoietic stem cell transplants by eradicating malignant cells, known as the graft-versus-leukemia effect. Failure to eradicate all malignant cells can lead to leukemia relapse following marrow transplantation. The relapse rate is lower in patients who develop GVHD and higher in patients who receive T cell-depleted marrow, which suggests a T cell-mediated graft-vs-leukemia (GVL) effect. Further, complete remission of relapsed leukemia following hematopoietic stem cell transplantation may be achieved by donor lymphocyte infusion (DLI) taking advantage of the GVL effect. DLI has also been shown to eradicate residual recipient hematopoiesis following HLA-matched sibling transplantation for sickle cell disease. DLI is most effective in patients with CML relapse with complete remission rate of 70 to 80 percent compared to 20 to 30 percent for patients with AML. The response to DLI is lower with increased tumor burden and the balance between GVL effect in DLI and risk of GVHD is a dose-dependent; higher T-cell doses cause similar remission rates but with higher GVHD side effects.

Given the positive GVL effect of DLI, it has been postulated that the removal of T cells by leukocyte reduction of blood components may have a deleterious effect on malignancy recurrence. However, several reports have demonstrated no difference in disease-free survival among leukemia patients receiving either leukocyte-reduced or standard blood components. On the other hand, Oksanen and colleagues observed a favorable effect of leukocyte reduction on disease-free survival, hematopoietic recovery, and post-transplantation infection. Thus, leukocyte-reduced components may be used for their beneficial effects in hematopoietic stem cell transplant patients without adversely affecting malignancy recurrence.

Reperfusion Injury

The restoration of blood flow to ischemic myocardium following cardiac bypass surgery increases the structural and functional damage caused by anoxia alone. Ischemia promotes activation of neutrophils which bind to the endothelium and migrate into the underlying myocardial tissue with subsequent release of oxygen-free radicals and proteases. Accumulation of neutrophils may also impair restoration of blood flow in affected capillaries. The combination of these biochemical effects can decrease cardiac output, increase pulmonary vascular resistance and increase the risk of arrhythmia. Infusion of fructose-1,6-diphosphate, a high energy intermediate in the glycolytic pathway, and pharmacologic blockade of...
the endothelial or neutrophil receptors have been shown to attenuate the ultrastructural damage seen in reperfused organs. Thus, it has been suggested that the removal of leukocytes from reperfused blood may reduce the harmful affects of neutrophils on ischemic tissues.\textsuperscript{319,320} Currently available leukocyte reduction filters are capable of removing over 90 percent of the reinfused leukocytes.

Several studies have examined the effect of leukocyte reduction on myocardial reperfusion injury. In animal models, the use of leukocyte-reduced blood resulted in less histochemical damage, better endothelial function, decreased coronary vascular resistance with better myocardial blood flow, increased left ventricular pressure and output, and fewer arrhythmias.\textsuperscript{321-327} In heart transplant patients, Pearl and colleagues\textsuperscript{328,329} observed less ultrastructural damage, decreased enzyme release, and less inotropic support in patients reperfused with leukocyte-reduced blood. In cardiac bypass, removal of leukocytes in reperfused blood is also effective in decreasing the biochemical markers of myocardial damage, including creatine kinase MB, troponin-T, thromboxane B\textsubscript{2}, and malondialdehyde.\textsuperscript{329-336} In addition, leukocyte reduction was associated with better ejection fraction, increased cardiac output and fewer ventricular arrhythmias.\textsuperscript{330,332,336}

Leukocyte removal may also improve pulmonary function, as fewer neutrophils are sequestered or activated in the lungs of dogs when filtered blood is used.\textsuperscript{337} In a randomized prospective study, Olivencia-Yurvati and colleagues\textsuperscript{317} found lower pulmonary microvascular pressures and decreased pulmonary shunting in patients receiving leukocyte-reduced blood. Patients in the treatment group also had lower hospital charges and length of stay. Although Gu et al.\textsuperscript{338} observed improved pulmonary gas exchange in postoperative cardiac patients receiving leukocyte-reduced reperfusionate, two other reports failed to demonstrate a positive effect on pulmonary function.\textsuperscript{339,340} Thus, there is increasing evidence that leukocyte reduction in the cardiac bypass circuit reduces myocardial reperfusion injury, it remains to be determined if leukocyte reduction improves pulmonary function in cardiac bypass patients.

**SUMMARY AND RECOMMENDATIONS**

In almost all clinical situations, allogeneic leukocytes contained in blood components do not convey any benefits to transfusion recipients, yet these cells are associated with many of the adverse outcomes to transfusion. Febrile reactions occur less frequently when sensitized patients receive leukocyte-reduced components. Leukocytes facilitate primary alloimmunization and lead to refractoriness in patients who have not been pregnant previously or sensitized otherwise. Removing leukocytes from blood components for these patients may have clinical, and even financial benefits, but leukocyte reduction for previously sensitized patients may provide only an illusion of benefit. Leukocyte reduction appears to be as effective as the use of CMV-seronegative components in avoiding CMV transmission. Given that many of the patients requiring CMV-reduced-risk components also may benefit from avoiding alloimmunization, two benefits may be achieved for a single expenditure.

The possibility of avoiding immunomodulation through leukocyte reduction of allogeneic components is intriguing. If the benefits of leukocyte reduction for reducing transfusion-related immunomodulation can be demonstrated conclusively, this would provide strong support for the implementation of universal leukocyte reduction. The additional filtration cost would be overwhelmed by this savings achieved from avoiding the complications of this effect. However, demonstration of such benefits in clinical trials has proven difficult.

The lack of complete data precludes a refined analysis of the outcomes of applying leukocyte reduction in each of the above clinical situations. This hinders firm conclusions regarding the most appropriate applications of this technology. As the consequences of exposure to allogeneic leukocytes are usually not perceived as life threatening, many clinicians would prefer to wait for definitive proof of benefit rather than advocating universal leukocyte reduction given the added cost. Others who are persuaded that the available data make a sufficiently strong case regarding the immunosuppressive consequences of allogeneic exposure, find a rationale to provide all or nearly all transfusions as leukocyte reduced.

While the clinical indications for leukocyte reduction remain debatable at present, the optimal timing of the removal of passenger leukocytes has been shown clearly to be prior to storage. Not only does pre-storage leukocyte reduction allow for better quality assurance, but also removal before the
fragmentation of leukocyte membranes and the production of pyrogenic and immunomodulatory cytokines can be achieved. Therefore, while the “if” question remains for leukocyte reduction, the “when” has a much clearer answer.

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Leukoreduction of Blood Components


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Leukoreduction of Blood Components


PLATELET MORPHOLOGY

Light Microscopy

Platelets are small fragments of the cytoplasm derived from megakaryocyte. In the circulation or in freshly prepared samples from human blood, they have a characteristic discoid form 3 to 4 μm in diameter. The platelets in peripheral blood are heterogeneous with respect to size, density and staining characteristics. Their size generally varies from 2 to 4 μ in diameter and average 5 to 8 fl in volume. The morphology also varies greatly depending on the methods by which they are examined, the anticoagulant employed and the temperature during collection and processing. In wet preparations, platelets appear as colorless, moderately refractile bodies that are discoid or elliptical. Under dark field illumination, they look translucent and reveal a sharp contour. A few immobile granules are present in the center of the cell. In polychrome-stained blood smears, platelets appear round, oval or rod shaped.

PLATELET ULTRASTRUCTURE

Platelets in the quiescent state have smooth, convex surface contours. On closer inspection, however, random indentations are apparent on the platelet surface, representing sites of communication between channels of the surface-connected or open canalicular system and the exterior of the cell. Figure 6.1 represents a schematic diagram of the platelet. In order to relate structure to function, White has divided the anatomy of the platelet into distinct zones. The peripheral zone consists of membranes and closely associated structures. The platelet plasma membrane has a fuzzy coat (glycocalyx), a dense layer 150 to 200Å in thickness and is rich in glycoproteins (GPs). Biochemical studies have shown that the exterior coat of platelets contains at least ten different glycoproteins (GPs Ia, Ib, Ic, IIa, IIb, IIIa, IV, V, VI and IX) (Wintrobe, 1999), which can be specifically radiolabeled and purified by polyacrylamide gel electrophoresis. They belong to different gene families and accordingly identified in three groups: (a) integrins comprised of GPIa, Ic, and the IIb/IIIa complex, (b) leucine-rich GPs Ib, IX and V, and (c) the other GPs belonging to the 7-transmembrane domain family. The middle layer of the peripheral zone is rich in phospholipids. Major lipids include: phosphatidylcholine (26.3%), phosphatidylserine (6.6%), sphingomyelin (11.6%), phosphatidylethanolamine (8.6%), and cholesterol (30.8%), phosphatidylinositol (2.7%). The phospholipids are arranged in a bilayer with their polar head groups oriented to the external or internal aqueous environment and their long acyl chain oriented perpendicular to the plane of the membrane and forming a hydrophobic core.

The phospholipids are arranged asymmetrically within the bilayer. Cholesterol is solubilized by the phospholipids and modulates the fluidity of the bilayer. Interspersed within this asymmetric and fluid lipid matrix are certain glycoproteins, which serve as receptors for agonist/surface-mediated stimuli involved in triggering platelet activation.

The sol-gel zone is the matrix of the platelet cytoplasm. It contains fiber systems in various states of polymerization which support the discoid shape in resting platelets and provide a contractile system involved in shape change, pseudopod extension,
contraction and secretion. The contractile system constitutes 30 percent of the total platelet proteins. A significant portion of this system is actin. Other proteins of the platelet contractile system include myosin, tropomyosin, actin-binding protein, α-actinin, gelsolin, profilin, vinculin and spectrin. The term cytoskeleton is frequently used to describe this zone or its detergent-resistant elements.

The alpha granules, dense bodies, peroxisomes, lysosomes, mitochondria and glycogen together form the organelle zone. This zone serves as the storage site for various enzymes, nonmetabolic adenine nucleotides, serotonin, a variety of proteins, calcium and antioxidants such as ascorbic acid, glutathione and taurine. Alpha granules are the most numerous of the organelles in the platelet cytoplasm and can be clearly differentiated from the mitochondria and electron dense bodies. They are about 50 to 80 per platelet. It has a central electron dense nucleoid in which β-thromboglobulin, platelet Factor IV, and proteoglycans are stored. The other contents include von Willebrand’s factor (vWF), fibrinogen, thrombospondin, Factor V, fibronectin, plaminogen activator inhibitor (PAI)-1, α, antiplasmin and PDGF. Electron dense bodies are about 200 to 300 nm in diameter, are distinctive because of their intensely opaque internal content, which was often separated from the enclosing membrane by a clear space. It contains mostly a pool of adenine nucleotides, calcium and magnesium and serotonin [5-hydroxytryptamine (5HT)]. Mitochondria are easily differentiated from other structures by the plication of their internal membranes into cristae. Glycogen forms an essential component and is the seat of platelet metabolism along with mitochondria.

**PLATELET ORIGIN AND DEVELOPMENT**

Megakaryocytopoiesis, the process of development of platelets, is regulated by a mechanism that depends upon developing progenitors that become progressively committed to a single megakaryocytic lineage. Megakaryocytes are derived from pluripotent stem cell, the earliest recognized form being burst-forming unit called BFU-meg. Under the influence of thrombopoietin (Tpo) and cytokines like interleukin (IL)-11, IL-3, the BFU-meg develop into colony-forming units-meg (CFU-meg) which undergo endomitotic reduplication and mature into megakaryoblasts and then into megakaryocytes. Their ploidy state progresses from 2 N to 32 N or even 64 N. The cellular size also increases from 6 to 20 microns to 60 microns as well as there is the development of platelet granules and membrane glycoproteins which is vital to platelet functions. These fully mature megakaryocytes are so situated in the bone marrow in relation to the blood vessels that small projections or moderately long pseudopods are easily pinched off by the force of blood flow and carried into the circulation. By such a process, the whole cytoplasm of the megakaryocyte is broken away giving rise to individual platelets or larger cytoplasmic fragments called proplatelets. The latter is transported through the bloodstream to the lungs via the heart where final mechanical fragmentation is accomplished by the pulmonary microcirculation giving rise to about 3,000 platelets from each megakaryocyte.

The normal lifespan of platelet ranges between 8 and 10 days. In healthy subjects, the platelet survival curve is linear, indicating the removal of platelets as a result of senescence rather than random utilization. Effete platelets are taken up by the reticuloendothelial system in the liver, spleen and bone marrow. The normal platelet count being 150 to 400 × 10⁹ /L, which is maintained by negative feedback mechanism between circulating platelet count and thrombopoietin level.

**PLATELET FUNCTION**

The platelets under normal physiological conditions circulate in the blood vessels as single cells and do
not interact with other platelets. However, when exposed to an appropriate stimulus, they undergo a transition from the nonadhesive to an adhesive state. It is seen that disruption of the endothelial cell lining of the blood vessels exposes constituents of the subendothelial matrix, including a variety of adhesive proteins that can support initial platelet attachment. Following attachment, the platelets undergo a spreading reaction forming multiple contacts between the cell surface and the matrix. In conjunction with these adhesive reactions, the cells also encounter agonists that trigger platelet secretion within the microenvironment, which leads to the release of intracellular storage granule contents. These secreted granules stimulate other circulating platelets and cause them to acquire new adhesive properties. The interaction of these stimulated platelets with one another leads to aggregation and formation of an effective platelet plug that seals the injured vessel wall and prevents blood loss. The sequential platelet responses, viz. attachment, spreading, secretion and aggregation, are essential for the hemostatic function of platelets.

**Adhesion**

Platelets in the normal course of events are prevented from adhering to endothelial cells (ECs), due to the high concentration of prostaglandin I₂ (PGI₂) and endothelium-derived relaxing factor (EDRF), which bind to specific receptors on the platelet membranes. Platelets escaping from injured vessel wall come into contact with, and adhere to vessel wall components. Collagen, vWF, fibronectin, thrombospondin, laminin and microfibrils are some of the subendothelial matrix proteins of the vessel wall that are involved in the attachment of platelets to the endothelium. The endothelium also creates an effective barrier to prevent circulating platelets from reaching the matrix and initiating thrombus formation. In addition to serving as a physical barrier, endothelial cells synthesize and develop components such as PGI₂, which prevent platelet activation and impart a non-thrombogenic character to the normal endothelium. Coverage of the exposed site by platelets depends on the recognition of adhesive proteins by platelet-membrane glycoproteins. Glycoprotein Ib receptor (a nonintegrin), exists as a complex with glycoprotein IX and V on the platelet surface and binds vWF. It is through this receptor that the initial contact between the platelets and the endothelium occurs and vWF is known to form the bridge between the two. Glycoprotein IIb/IIIa (an integrin; \(\alpha_{IIb}\beta_{3}\)) is known to have dual function and is involved in both platelet adhesion and aggregation. The other platelet integrin GP Ia/IIa (\(\alpha_{2}\beta_{1}\)) has been shown to be the principal receptor involved in binding of platelets to collagen. However, GP IV, a nonintegrin, has also been shown to play a role in platelet-collagen interaction as well as interaction of platelets with thrombospondin. Glycoprotein Ic/IIa (\(\alpha_{6}\beta_{1}\)), a fibronectin receptor, \(\alpha_{6}\beta_{1}\), a laminin receptor, and \(\alpha_{6}\beta_{3}\), a vitronectin receptor, are some of the other integrins that contribute to platelet adhesion.

**Shape Change**

Platelet shape change occurs as response to many different agonists. They change from the normal discoid shape and transform to spiny spheres with long, thin pseudopodia extending several mm in size. They are the sites of establishing contact and enhance interaction with adjoining platelets. This shape change is accompanied by reorganization of the internal constituents like microfibrils, microtubules and thereby forcing the granules towards the plasma membrane. Contraction of these microfibrils may account for the property of clot retraction.

**Secretion**

Platelets contain four types of granules: \(\alpha\) granules, dense or \(\delta\) granules, lysosomes and microperoxisomes. Following platelet stimulation, the contents of these granules are extruded from the platelet interior by a process known as platelet secretion. This secretory function of platelets requires energy from the metabolically active cellular stores of adenosine triphosphate (ATP). Adenosine diphosphate (ADP) in the granules is released into the plasma, stimulating further aggregation. Fibrinogen, vWF and other coagulation and adhesion proteins also are released, providing a further stimulus for aggregation and adhesion. Platelet specific proteins such as platelet factor-4 (PF-4) and \(\beta\)-thromboglobulin are also released. PF-4 is secreted as a complex with a
molecular weight of approximately 78,000 daltons and can bind and neutralize the anticoagulant activity of heparin. Although it has been detected in the vascular wall following endothelial trauma, its physiologic function is undetermined. β-thromboglobulin is an 88,000 daltons protein whose amino acid sequence is approximately 50 percent identical to that of PF-4 and exists as a tetramer. β-thromboglobulin is a low affinity antiheparin protein. It is stored in the α granules; and when secreted, it binds to endothelial cell membranes and inhibits prostacyclin secretion. Thus, this protein has been implicated in the pathophysiology of thrombosis. Levels of PF-4 and β-thromboglobulin in plasma have been used as markers of platelet activation.

Aggregation
A variety of substances including ADP, thromboxane A$_2$ (TXA$_2$), adrenaline, 5-HT, vasopressin and platelet-activating factor (PAF)-induced platelet aggregation. The layer of platelets adherent to exposed subendothelium provides the foundation for a hemostatic platelet plug. The plug is formed as the platelets stick specifically to one another in a process called platelet aggregation. Aggregation requires active platelet metabolism and prior platelet stimulation by one or more specific agonists. They appear to operate via a common pathway, wherein binding to specific but poorly defined receptors, is followed by signal transduction process. The GP IIb/IIIa complex is activated in the process, which permit fibrinogen binding followed by aggregation. The fibrinogen binding involves at least three peptides, two on the α chain and the third on the γ chain. Other adhesive proteins like fibronectin, vWF and thrombospondin could also operate in the similar fashion via their own specific binding sites. Two waves of platelet aggregation have been described; the first wave of primary aggregation being a direct consequence of agonist stimulation. It is reversible and does not associate with platelet secretion. The second wave or secondary aggregation occurs only after the platelets secrete their granular content leading to the formation of large irreversible platelet aggregates. The platelet aggregation, however, caused, leads to activation of and release from other platelets, setting up a self-sustaining cycle, which results in the formation of the platelet plug at the site of injury.

NEW ADVANCES IN MEASURING PLATELET FUNCTION
There are several new advances in measuring platelet function such as thromboelastography and sonoclot which are described below.

Thromboelastography and Sonoclot
Thromboelastography and sonoclot are qualitative tests of whole-blood coagulation that measure alterations in shear elasticity and mechanical impedance respectively produced by the changing viscoelastic properties of forming blood clot. The viscoelasticity of clotting blood increases with fibrin formation, platelet fibrin interaction and clot retraction, and it is decreased by clot lysis. Thus, characteristic tracings produced and their derived parameters in the presence of a quantitative or qualitative platelet deficiency may depict abnormalities in procoagulant proteins, platelets and fibrinolytic activity. Inadequate platelet number or function has characteristic abnormal sonoclot findings.

Thromboelastography and sonoclot have been shown to be more accurate predictors of postoperative damage after cardiac operation than other routine coagulation tests.

Lumiaggregometry
When the concentration of ATP is limiting, ATP secretion can be measured quantitatively by luminescence generated from the ATP-dependent firefly luciferin-luciferase reaction. Thus, the turbidometric platelet aggregometer has been modified to use the luciferin-luciferase reaction to measure ATP secretion and platelet aggregation from the same sample of platelet-rich plasma (PRP) simultaneously. Lumiaggregometry is particularly useful in evaluating patients with congenital or acquired disorder of platelet secretion.

Impedance Platelet Aggregometry
There is increase in electrical impedance as platelet aggregates accumulate on a pair of electrodes inserted into a suspension of aggregating platelets. In this method, whole blood can be used to measure platelet function. The advantages of impedance aggregometry are its ability to detect platelet aggregation in whole blood without the need to prepare PRP and in the presence of lipemic plasma.
**Single Platelet Counting**

Because turbidometric and impedance aggregometry are insensitive to the formation of smaller platelet aggregates, they cannot record the early events that follow platelet stimulation; therefore, methods based on the disappearance of single platelets following platelet stimulation being devised to the study the early events. Agonists are added to samples of PRP or whole blood and the number and size of the platelet aggregates that form are measured after sample is diluted with buffer containing fixatives such as glutaraldehyde or paraformaldehyde to stabilize the aggregates. Aggregate number and size have been determined visually or measured with an electronic particle counter or flow cytometer. Single platelet counting indicates that within 10 seconds of platelet stimulation, up to 90 percent of platelets are present in aggregates that may contain up to 100 platelets. Moreover, additional platelets are not recruited into aggregates during the second wave of aggregation.

**PLATELET PROCOAGULANT ACTIVITY**

The platelet-derived procoagulant activity falls into two main groups. One of the groups involves contact factors especially factor XI, while the other is induced by platelet membrane phospholipids. The phospholipid-mediated, Ca++ dependent procoagulant activity is generated at two stages of blood coagulation, and one involves factors IXa, VIII, and X, now called Factor Xa-forming activity or tenase. The other, which was previously termed platelet factor-3 (PF-3), now referred to as prothrombinase activity. The participation of platelets in the activation of factors X and V and prothrombin has been termed PF-3. This activity becomes available coincident with platelet secretion. PF-3 is required in at least 2 steps in the process of blood coagulation, namely the activation between factors IXa and VIII, which results in the activation of factor X as well as in the interaction between factor Xa and factor V, which leads to the formation of prothrombinase. PF-3 is a thermostable lipoprotein. When activated by ADP and other agonists under various in vitro conditions, PF-3 remains closely associated with the platelet membrane.

**Phospholipid-mediated procoagulant activity:**
Intact platelets have no intrinsic procoagulant activity; however, within seconds of the onset of aggregation, and to a lesser extent adhesion, a reorientation of the membrane phospholipid (PL) occurs, whereby the hydrophobic, negatively charged PLs are exposed on the surface.

**PLATELET MEMBRANE GLYCOPROTEINS**

The platelet plasma membrane like that of other cells is rich in glycoproteins that play an important role in its interactions with the environment. Since the primary function of the platelet in hemostasis involves its adhesion to the subendothelium of damaged vessels and its aggregation with other platelets, the major glycoproteins on the platelet surface are receptors for adhesive proteins. Similarly, the platelets also have receptors for important physiological stimulators such as thrombin, ADP, collagen, PAF and epinephrine.

There are at least 30 distinguishable glycoproteins in the platelet plasma membrane, but only seven of these predominate and are referred to by Roman numerals which increase with decreasing molecular mass. A detailed description of some of the glycoproteins is shown in Table 6.1. Two of these glycoproteins, i.e. GP Ib-IX and GP IIb-IIIa complex are abnormal in some inherited, bleeding disorders like Bernard-Soulier syndrome and Glanzmann’s thrombasthenia respectively.

**Glycoprotein IIb/IIIa (Receptor/CD 41a)**

The first tentative identification of GP IIb and GP IIIa as the platelet fibrinogen receptor came from early studies performed with platelets from patients with thrombasthenia. The GP IIb-IIIa complex is the most abundant and best characterized of the major platelet glycoproteins. A normal single platelet contains approximately 40 to 80,000 receptors on the surface and 20 to 40,000 receptors inside platelets. Besides being a major plasma membrane constituent, the GP IIb-IIIa complex is also present in the α-granule membrane. In addition to fibrinogen, the GP IIb-IIIa complex has been shown to bind vWF, thrombospondin and fibronectin. The GP IIb-IIIa receptor (α_{II}β_{3}) is a typical integrin. Its 136 Kd α-subunit, is a transmembrane protein with four calmodulin-like domains that are able to bind divalent cations. The mature protein has 1,008 amino acids,
with one transmembrane domain. The 92 Kd β-subunit consists of a single polypeptide of 762 amino acids, with a short cytoplasmic tail, a single transmembrane region and a large extracellular domain. The subunits are noncovalently bound to each other and calcium is required to maintain the heterodimer structure.

The fibrinogen recognition specificity of the GP IIb-IIIa receptor is defined by two peptide sequences. The Arg-Gly-Asp (RGD) sequence was initially identified as the adhesive sequence in fibronectin but is also present in fibrinogen, vWF and vitronectin. All these ligands contain at least one RGD sequence, whereas fibrinogen contains two RGD sequences, one near the carboxy terminus of each of the two αA chains (amino acid 572–574 and another at amino acids 95-97). In addition, the carboxy terminal 12 amino acids of each of the γ chains (amino acids 400–411) contain a sequence that includes Lys-Gln-Ala-Gly-Asp-Val, and it appears that the Lys and Gly-Asp form a molecular mimic of the RGD sequence (Hawiger 1991). The major sequence involved in the binding of fibrinogen to GP IIb-IIIa receptors is the Lys-Gln-Ala-Gly-Asp-Val sequence located at the carboxy terminus of the γ chain of fibrinogen and is probably the predominant site for the binding of fibrinogen to glycoprotein IIb-IIIa receptors; RGD sequence may also participate. Small synthetic peptides containing the RGD or γ-chain sequence inhibit binding of fibrinogen to platelets, this observation has been used to develop therapeutic agents to inhibit platelet thrombus formation and monoclonal antibodies as antiplatelet agents.

Platelet aggregation measured in the aggregometer ex vivo depends upon fibrinogen binding to GP IIb/IIIa. Other glycoproteins which contain RGD sequence and can bind GP IIb/IIIa on activated platelets include vWF, fibronectin, vitronectin and thrombospondin.

A schematic diagram of the GP IIb-IIIa receptor has been shown in Figure 6.2.

GP IIb and IIIa also contain the antigens responsible for most cases of post-transfusion purpura and neonatal thrombocytopenia. The larger subunit of IIb contains the Bak (Lek) antigen, while the P1A antigens are located on the IIIa component. The complete amino acid sequences of IIb and IIIa have been deduced from the nucleotide sequences of IIb and IIIa cDNA. Because human erythroleukemia

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>Mass (KD) (Unreduced)</th>
<th>Subunits</th>
<th>Mass (KD) (reduced)</th>
<th>Chromosome location</th>
<th>Number*</th>
<th>Receptor for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>150</td>
<td>α3</td>
<td>5</td>
<td>5000</td>
<td>1000</td>
<td>Collagen</td>
</tr>
<tr>
<td>Ib</td>
<td>170</td>
<td>α</td>
<td>145</td>
<td>1</td>
<td>5000</td>
<td>vWF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>22</td>
<td>22</td>
<td>25000</td>
<td>vWF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bThrombin</td>
</tr>
<tr>
<td>Ic</td>
<td>140</td>
<td>α6</td>
<td>2</td>
<td>1000</td>
<td></td>
<td>Laminin</td>
</tr>
<tr>
<td>IIa</td>
<td>138</td>
<td>β1</td>
<td>148</td>
<td>10</td>
<td>1000</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>IIb</td>
<td>145</td>
<td>α</td>
<td>125</td>
<td>17</td>
<td>60000</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>23</td>
<td></td>
<td>50000</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>IIIa</td>
<td>90</td>
<td>β3</td>
<td>114</td>
<td>17</td>
<td>50000</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fibronectin</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calcium</td>
</tr>
<tr>
<td>IV</td>
<td>88</td>
<td></td>
<td>7</td>
<td>20000</td>
<td></td>
<td>Collagen,</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>TSP</td>
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<tr>
<td>V</td>
<td>82</td>
<td></td>
<td>82</td>
<td>?</td>
<td>12500</td>
<td>Thrombin</td>
</tr>
<tr>
<td>VI</td>
<td>62</td>
<td></td>
<td>62</td>
<td></td>
<td></td>
<td>Collagen</td>
</tr>
<tr>
<td>IX</td>
<td>17</td>
<td></td>
<td>17</td>
<td>3</td>
<td>25000</td>
<td>vWF,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thrombin</td>
</tr>
</tbody>
</table>

Table adapted from Barry S Coller 1995

a. Number per platelet
b. von Willebrand 2 factor binds to GP Ib on unstimulated platelets and to the GP IIb-IIIa complex on stimulated platelets.
c. Number depends on experimental conditions
d. GP V is a (prototype) substrate for thrombin (McGregor et al 1981) and it has been detected under reducing conditions in Bernard-Soulier platelets (Peterson 1982).
(HEL) cells and endothelial cells constitutively express IIb-IIIa and IIIa respectively, the cDNAs for these were isolated from HEL cell and endothelial cell cDNA libraries. Recently, partial sequences for platelet IIIa were obtained by amplifying residual platelet ribonucleic acid (RNA) using polymerase chain reaction (PCR). The sequences were found to be identical to the HEL and endothelial cell sequences. GP IIb/IIIa is also suggested to be involved in the binding of plasminogen, Factor XIIIa, immunoglobin E (IgE) binding leading to parasite cytotoxicity and calcium transport across platelet plasma membrane.

**Glycoprotein Ib-IX Receptor/CD42**

When blood vessels are damaged exposing the subendothelial connective tissue matrix to circulating blood, platelets adhere to the subendothelial matrix thus providing the hemostatic plug. Under high shear conditions, as seen in arterioles and in microcirculation, this adhesion is initiated by the binding of the platelets to the vWF through the GP Ib-IX receptor.

The GPIb-IX complex is composed of four glycoprotein subunits GP Ibα, GP Ibβ, GP IX and GP V, each with a variable number of leucine-rich repeats. GP Ibα and GP Ibβ are linked by disulfide bridge, but the GP Ib and GP IX are associated with each other through non-covalent bonds.

Also, GP V is known to be associated with the GP Ib through non-covalent binding. The GP Ib-IX complex is formed by a 1 : 1 non-covalent association of the 170,000 dalton GP Ib, with the 17,000 dalton GP IX. The GP Ibα chain has a molecular weight of 143,000 daltons with 610 amino acids, whereas the GP Ibβ component has a molecular weight of 23,000 daltons with 122 amino acids.

The complex is embedded in the platelet membrane and the cytoplasmic portion of Ib is associated with the platelet cytoskeleton by its interaction with the actin-binding proteins (Ezzell et al, 1988). Each platelet contains about 25,000 copies of the GP Ib-IX receptor. Figure 6.3 shows a schematic diagram of the
Platelets in Health and Disease

Pathophysiological Classification of Thrombocytopenias

1. Decreased platelet production
   a. Hypoplasia of megakaryocytes
   b. Ineffective thrombopoiesis
   c. Disorders of thrombopoietic control
   d. Hereditary thrombocytopenias

2. Increased platelet destruction
   a. Caused by immunologic process
      i. Autoimmune
         1. Idiopathic thrombocytopenic purpura (ITP)
         2. Secondary: infections, pregnancy, drugs, lymphoproliferative disorders, collagen vascular disorders
      ii. Alloimmune
         1. Neonatal thrombocytopenia
         2. Post-transfusion purpura
   b. Caused by non-immunologic process
      i. Thrombotic microangiopathies
         1. Disseminated intravascular coagulation (DIC)
         2. Thrombotic thrombocytopenic purpura (TTP)
      3. Hemolytic uremic syndrome (HUS)
   ii. Platelet damage by abnormal vascular surfaces
   iii. Miscellaneous
      1. Infection
      2. Massive blood transfusion

Table 6.2: Platelet glycoproteins and granules

<table>
<thead>
<tr>
<th>Platelet membrane glycoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>• GP Ib-IIIa: fibrinogen receptor, critical for platelet aggregation</td>
</tr>
<tr>
<td>• GP Ib-IX-V: von Willebrand factor receptor: critical for initial platelet adhesion to subendothelium</td>
</tr>
<tr>
<td>• GP Ia-IIa, GP VI: collagen receptor, involved in initial platelet adhesion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platelet granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Alpha granules: contain proteins that contribute to platelet adhesion, aggregation and coagulant activity</td>
</tr>
<tr>
<td>• Dense granules: contain ADP and calcium, which contribute to platelet aggregation and coagulant activity</td>
</tr>
<tr>
<td>• Lysosomes: contain acid hydrolases that may participate in thrombus degradation</td>
</tr>
</tbody>
</table>

ADP = adenosine diphosphate

GP Ib-IX complex. GP Ib has been implicated as the target antigen in autoimmune thrombocytopenia and in quinine and quinidine-induced thrombocytopenia. The function of GP IX is unknown, but it is probably required for the efficient expression of GP Ib. Platelet interaction with vWF is mediated with GP Ib, at the region on vWF between amino acids 449 to 728. This region is distinct from the RGD region for GP Ib/IIa. Unlike GP Ib/IIa, which requires intact, activated platelets for binding, GP Ib-mediated binding does not require platelet activation, since fixed platelets are readily agglutinated in the presence of vWF and either ristocetin or botrocetin. This finding forms the basis of plasma vWF assay.

Much of the present-day knowledge about the role of GP Ib-IX complex has been obtained from the patients of Bernard-Soulier syndrome. These patients are known to lack the GP Ib-IX receptor due to an autosomal defect. The platelets from these patients fail to aggregate with ristocetin and vWF (Howard et al, 1973).

P-Selectin (CD 62-P)

P-Selectin, also called GMP-140, is an indicator of platelet activation (Coller, 1989). It is a glycoprotein present in the α granules of platelet and joins the plasma membrane when activated. It is also present in the Weibel-Palade body membranes of the endothelial cells. P-Selectin has a modular structure in which the amino terminal has calcium-dependent lectin domain and a cytoplasmic domain that binds carbohydrates. The gene is located on chromosome 1 and the Selectin family includes E-Selectin and L-Selectin as well. P-Selectin also mediates the attachment of neutrophils to platelets and endothelial cells. Increase in plasma levels of P-Selectin has been reported in thrombocytopenic platelet disorders (Chong et al, 1994) (Table 6.2).

PLATELET DISORDERS

Disorders of platelets emanate from both qualitative and quantitative defects and they manifest as thrombocytopenias or functionally inactive/defective platelets. They can be further classified as follows.
3. Abnormal platelet distribution/pooling
   a. Splenomegaly caused by congestive, neoplastic, infiltrative or infectious cause
   b. Hypothermia
   c. Dilution of platelets by massive transfusions

4. Artificial thrombocytopenia
   a. Giant platelets
   b. Platelet satellitism
   c. Pseudothrombocytopenia.

Few of the important disorders are dealt with in detail below, and the reader is advised to consult standard texts for further details on the other disorders.

THROMBOCYTOPENIAS

Idiopathic Thrombocytopenic Purpura

Idiopathic thrombocytopenic purpura (ITP), also known as immune thrombocytopenic purpura, is a common cause of thrombocytopenia in both adults and children. Patients may present with purpura and mucosal bleeding, or may be asymptomatic, and detected only by a routine blood count. There is no specific diagnostic study that defines ITP; the diagnosis can be made only by excluding other causes of thrombocytopenia. In children, the peak age of occurrence is 2 to 4 years, and the frequency is equal in boys and girls. In adults, most case series report that about 70 percent of patients are women, and 70 percent of these women are <40 years old. However, recent studies have described more cases in older patients and in men, perhaps a result of increased detection of asymptomatic patients by routine blood counts. Among children, ITP is typically acute in onset, with the occurrence of severe and symptomatic thrombocytopenia. Most children will recover even without specific treatment. Thrombocytopenia persists for more than 6 to 12 months in fewer than 20 percent of children, and even many of these children will experience a spontaneous remission during the ensuing years. Among adults, ITP is typically chronic, insidious in onset and, in some patients, refractory to treatment. However, the long-term natural history of ITP in adults is not well documented. Some reviews suggest a mortality of 5 percent, yet many patients have severe thrombocytopenia for many years with minimal symptoms. Most patients who are incidentally diagnosed with only mild or moderate thrombocytopenia appear not to develop severe, symptomatic thrombocytopenia.

Diagnosis

When the history, physical examination, and complete blood counts with examination of the blood smear are consistent with the diagnosis of ITP and do not suggest other causes of thrombocytopenia, few additional diagnostic studies are necessary as given in Table 6.3, which presents a differential diagnosis of thrombocytopenia, which is the foundation of the diagnosis of ITP. Symptoms and signs suggestive of other diagnoses should be excluded. These include the following:

i. a history of systemic signs and symptoms suggesting sepsis or thrombotic thrombocytopenic purpura-hemolytic uremic syndrome (TTP-HUS);
ii. splenomegaly suggesting disorders such as liver disease or lymphoma;
iii. anemia or neutropenia suggesting marrow failure; and
iv. the use of medicines or other remedies or drugs which may cause thrombocytopenia. Bone marrow examination may be important in older patients to rule out the presence of myelodysplasia or lymphoproliferative malignancies, particularly before splenectomy or potentially toxic treatments are considered. A test for human immunodeficiency virus (HIV) is important in patients with risk factors for HIV infection. Tests for platelet antibodies are not helpful, as both their sensitivity and specificity appear to be limited.

Table 6.3: Reasons to suspect hereditary thrombocytopenia

<table>
<thead>
<tr>
<th>Reason</th>
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<tbody>
<tr>
<td>1. Family history of thrombocytopenia, especially parent-child or maternal uncle-nephew</td>
</tr>
<tr>
<td>2. Lack of platelet response to autoimmune thrombocytopenia (AITP) therapies including IVIG, IV anti-D, steroids, and splenectomy and also immune-modulating treatments, e.g. azathioprine, rituximab</td>
</tr>
<tr>
<td>3. Diagnostic features on smear such as abnormal size of platelets (small, large, or giant); absence of platelet alpha granules (gray platelets); Döhle-like bodies (MYH9); or microcytosis (XLT-T)</td>
</tr>
<tr>
<td>4. Bleeding out of proportion to the platelet count</td>
</tr>
<tr>
<td>5. Onset at birth</td>
</tr>
<tr>
<td>6. Associated features such as absent radii, mental retardation, renal failure, high-tone hearing loss, cataracts, or the development of leukemia</td>
</tr>
<tr>
<td>7. Persistence of a stable level of thrombocytopenia for years</td>
</tr>
</tbody>
</table>
Differential Diagnosis of Idiopathic Thrombocytopenic Purpura

Falsely low platelet count
- **In vitro** platelet clumping caused by EDTA-dependent agglutinins
- Giant platelets.

Common causes of thrombocytopenia
- Pregnancy (gestational thrombocytopenia, pre-eclampsia)
- Drug-induced thrombocytopenia (common drugs include heparin, quinidine, quinine, and sulfa-namides)
- Viral infections, such as HIV, rubella, infectious mononucleosis
- Hypersplenism due to chronic liver disease.

Other causes of thrombocytopenia
- Myelodysplasia
- Congenital thrombocytopenia
- Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome
- Chronic disseminated intravascular coagulation.
- Autoimmune diseases, such as systemic lupus erythematosus (SLE)
- Lymphoproliferative disorders, such as chronic lymphocytic leukemia and non-Hodgkin’s lymphoma.

Management

In both children and adults, the goal of treatment is prevention of major bleeding, not cure of the ITP.

Initial Management of ITP in Children

Because spontaneous recovery is expected in children with ITP, some pediatric hematologists advocate only counseling and supportive care, rather than specific drug therapy. Others, however, advocate initial treatment with a short course of glucocorticoids, intravenous immunoglobulin, or anti-D, because randomized clinical trials have demonstrated that treatment may cause a more rapid recovery of the platelet count. However, there are no data that treatment diminishes the risk of major bleeding; therefore, it is uncertain if the benefits of treatment outweigh the risks of common side effects: behavioral problems with glucocorticoids, severe headache mimicking intracranial hemorrhage with intravenous immunoglobulin, and hemolysis with anti-D. Anti-D, though less expensive than intravenous immunoglobulin, is only effective in Rh(D)+ patients and is not effective in splenectomized patients. Recovery ultimately occurs in several weeks to months in 85 percent of children. Among the 15 percent of children with persistent thrombocytopenia, bleeding symptoms are uncommon and splenectomy is rarely required. However, splenectomy is an effective treatment option for children with severe and symptomatic thrombocytopenia, resulting in a complete remission in about 75 percent of children. The risk for overwhelming sepsis following splenectomy is greater in young children and, therefore, splenectomy should be deferred, if possible, until after 5 years of age. Immunizations for *Streptococcus pneumoniae* and *Haemophilus influenzae* should be given before splenectomy, and twice daily prophylactic penicillin should be prescribed following surgery.

Initial Management of ITP in Adults

Initial treatment with oral prednisone is standard care for adults with ITP, because it is assumed that the thrombocytopenia will be persistent. However, patients with only moderate thrombocytopenia and minimal symptoms may require no specific treatment. Therefore, deciding which patients to treat and which patients not to treat is the initial important management decision. Several case series suggest that it is appropriate to not treat patients with initial platelet counts over 20,000 to 50,000/ml. Some patients with mild-to-moderate thrombocytopenia may develop more severe thrombocytopenia and become symptomatic; some may experience a spontaneous remission; but in most, the moderate, asymptomatic thrombocytopenia will persist. Patients with symptomatic thrombocytopenia are treated initially with prednisone, 1 mg/kg daily. This increases the platelet count in most patients, but the platelet count often decreases again when prednisone is tapered or discontinued. Persistent or recurrent severe, symptomatic thrombocytopenia of 4 to 6 weeks’ duration is an indication for splenectomy. Splenectomy is the only treatment in adults with ITP that is associated with a high frequency of long-term remission. Most case series report sustained complete remissions in two-thirds of patients, and partial remissions in an additional 15 percent of patients. Recommendations
for immunization before splenectomy are the same as for children.

**Management of Children and Adults with Chronic, Refractory ITP**

Appropriate management is uncertain for adults and children who do not respond to splenectomy, or for children in whom splenectomy is deferred because of their young age. Many treatments have been advocated, often with optimistic preliminary data, yet none has been studied in a randomized, controlled clinical trial. Therefore, it is not possible to know if one treatment is better than another, or better than no treatment, or possibly worse than no treatment. For patients with severe and symptomatic thrombocytopenia, common recommendations are for cytotoxic or immunosuppressive regimens that may worsen thrombocytopenia. Common agents used in these patients, either alone or in combination, include cyclophosphamide, azathioprine, vinca alkaloids, rituximab, glucocorticoids, and danazol. Only a minority of patients will respond to any treatment. There are published anecdotes of success with removal of accessory spleens, but these reports are rare and mostly in children. In patients with refractory ITP, therefore, the only practical goal is the maintenance of a safe platelet count, possibly > 10,000–20,000/\(\mu l\), with minimal therapy. Although the perceived risk for bleeding may seem great, it is important that the morbidity from treatments does not exceed the morbidity of actual bleeding symptoms. Many patients have active, productive lives with persistent platelet counts <20,000/\(\mu l\).

**Emergency Treatment**

Management of major bleeding requires platelet transfusions in combination with high doses of parenteral glucocorticoid and/or intravenous gammaglobulin. Platelet count increments after a platelet transfusion may be substantial and sustained, especially when administered after intravenous gammaglobulin. Methylprednisolone (1 g or 30 mg/kg intravenously daily for 2–3 days) or intravenous gammaglobulin (1 g/kg for 1–2 days) usually increases the platelet count within several days. These are temporary measures; the platelet count typically returns to the pretreatment level 2 to 3 weeks after administration of intravenous gammaglobulin.

**Management of Women during Pregnancy, and of their Newborn Infants**

When ITP occurs during pregnancy, there is an additional concern that thrombocytopenia may occur in the newborn infant. The risk of clinically important thrombocytopenia in the fetus is negligible, in contrast to neonatal alloimmune thrombocytopenia. The frequency of platelet counts below 20,000/\(\mu l\) at birth is about 4 percent and of counts less than 50,000/\(\mu l\) is about 10 percent. Infants born to mothers who have had more severe ITP which had required splenectomy or with severe thrombocytopenia during pregnancy may be at greater risk for thrombocytopenia. There are no data indicating that delivery by cesarean section is safer for the infant than routine vaginal delivery. Therefore, current practice is to manage the pregnancy and delivery according to standard obstetric indications. An important consideration is that infants born to mothers with ITP will often experience their most pronounced decrease in their platelet count several days after birth.

**Gestational Thrombocytopenia**

Approximately 5 percent of normal women with uncomplicated pregnancies have mild thrombocytopenia at the time of labor and delivery. The etiology of gestational thrombocytopenia (also called incidental thrombocytopenia of pregnancy) is unknown, but the clinical characteristics are mild thrombocytopenia occurring late during the pregnancy that resolves spontaneously after delivery and is not associated with fetal thrombocytopenia. There are no specific diagnostic tests that define gestational thrombocytopenia; the diagnosis requires exclusion of other causes of thrombocytopenia. As with ITP, tests for antiplatelet antibodies are not helpful. The most important diagnostic clue in differentiating ITP from gestational thrombocytopenia is a history of previous thrombocytopenia occurring late during the pregnancy that resolves spontaneously after delivery and is not associated with fetal thrombocytopenia. There is no evidence indicating that delivery by cesarean section is safer for the infant than routine vaginal delivery.
Thrombocytopenia with Infection

Mild and transient thrombocytopenia predictably occurs with many systemic infections. Thrombocytopenia may be caused by a combination of mechanisms: decreased production, increased destruction, and increased splenic sequestration. In some infections, specific mechanisms may predominate. In viral infections, platelet production may be suppressed; in rickettsial infections, platelets may be consumed in vasculitic lesions; in bacteremia, platelets may be consumed because of disseminated intravascular coagulation (DIC). Thrombocytopenia is commonly associated with HIV infection. Platelet kinetic studies in patients with HIV infection show that decreased platelet production occurs despite the presence of normal numbers of normal-appearing marrow megakaryocytes. Thrombocytopenia in HIV infection is typically mild and usually requires no specific treatment.

Drug-induced Thrombocytopenia

Unexpected thrombocytopenia in adults is often caused by drugs; drug-induced thrombocytopenia is rare in children. With so many drugs reported to cause thrombocytopenia, the decision of which drugs to discontinue is difficult. A case-control study of patients with acute reversible thrombocytopenia compared to patients with no thrombocytopenia demonstrated that the greatest difference for drug use was for quinine and quinidine, followed by sulfonamides and sulfonylurea agents. Because of its common use, the drug most commonly associated with thrombocytopenia in this study was trimethoprim-sulfamethoxazole. A systematic review of individual patient data also documented that the most commonly reported drugs with a definite or probable causal relation to thrombocytopenia were quinidine, quinidine, rifampin, and trimethoprim-sulfamethoxazole. This review found that for many reports of drug-induced thrombocytopenia, the evidence for a causal relation was weak. Complete data for all 690 English language articles describing 921 patients with assumed drug-induced thrombocytopenia is available on the website http://moon.uhsc.edu/jgeorge. The diagnosis of drug-induced thrombocytopenia is supported by recovery to a normal platelet count in 5 to 7 days. The diagnosis may be difficult if the patient and physician do not recognize that beverages and nonprescription remedies may contain quinine, or that herbal medicines and certain foods may cause acute thrombocytopenia.

The platelet GP IIb-IIIa antagonists, used as antithrombotic agents for acute coronary syndromes, can cause acute, profound thrombocytopenia with the initial treatment in about 1 percent of patients. This is unique, since all other drugs require prior exposure to cause sensitization and drug-dependent antibody formation. Therefore, it must be assumed that these patients have “naturally occurring” antibodies to neoepitopes on GP IIb-IIIa exposed by these agents. This mechanism is comparable to the “naturally occurring” platelet agglutinins causing pseudothrombocytopenia in EDTA anticoagulated blood.

Treatment of drug-induced thrombocytopenia may only require withdrawal of the offending drug. Often, prednisone is given, since the diagnosis of ITP cannot be excluded. Patients with severe thrombocytopenia caused by GP IIb-IIIa antagonists may require platelet transfusions because they are typically also receiving heparin and aspirin for their acute coronary disease. Although assays for drug-dependent antibodies exist, the results are not available quickly enough to make initial management decisions. Also, the sensitivity and specificity of tests for drug-dependent antibodies are unknown.

Heparin-induced Thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is a common drug-associated thrombocytopenia in hospitalized patients. It is distinct from other drug-induced thrombocytopenias because the major problem is thrombosis, not bleeding. There are two types of HIT. The first is a modest, transient decrease in the platelet count that occurs soon after starting heparin, caused by heparin-induced platelet agglutination. The platelet count may return to normal while heparin is continued. The more serious form occurs in about 1 percent of patients receiving unfractionated heparin. In these patients, there is a 50 percent or more reduction in the platelet count that usually begins 5 or more days after starting heparin therapy. Patients with previous heparin exposure may develop thrombocytopenia earlier. It is important to recognize that HIT can develop 1 to 2 weeks after stopping heparin.
Severe thrombocytopenia with bleeding is rare; the major problem is a prothrombotic state. One-third of patients may develop venous or arterial thrombosis, particularly if they have other risk factors for thrombosis, such as recent surgery. HIT is triggered by antibodies directed against heparin complexed to PF-4. Heparin binds to platelets, causing them to secrete PF-4 and resulting in the formation of heparin-PF-4 complexes on the platelet surface. When antibodies are formed to heparin-PF-4 complexes, they bind through their Fab portion and trigger activation of adjacent platelets by reaction with Fc receptors. Platelet activation can lead to the generation of procoagulant microparticles that contribute to the prothrombotic state. The immunoassays to detect antibodies against heparin-PF-4 complexes are of limited value since many patients undergoing cardiac surgery develop antibodies against the heparin-platelet factor 4 complex, yet few develop thrombocytopenia. More sensitive tests that measure platelet release of serotonin as an index of heparin-dependent, antibody-induced platelet activation are not commercially available.

It is important to discontinue heparin in patients with suspected HIT. Patients who were receiving heparin for treatment of arterial or venous thrombosis should be given an alternative anticoagulant agent. Low-molecular-weight heparins (LMWHs) cannot be used because most heparin-dependent antibodies also react with LMWHs. Instead, an anticoagulant such as danaparoid sodium (a mixture of heparan sulfate, dermatan sulfate, and chondroitin sulfate) or a direct thrombin inhibitor (such as hirudin or argatroban) should be given. Warfarin should not be started until adequate anticoagulation with these agents has been achieved, because protein C and S levels are further lowered by the warfarin therapy, and this may increase the risk for thrombosis. In patients with a remote history suggesting HIT, heparin may still be the best anticoagulant agent for a short procedure, such as coronary artery bypass surgery.

Inherited Thrombocytopenia

Inherited thrombocytopenias are uncommon; but when they occur, they are commonly misdiagnosed as ITP. Their recognition is important to avoid unnecessary and potentially harmful treatments. The consideration of inherited thrombocytopenia is especially important in children and young adolescents with a presumptive diagnosis of chronic ITP. Some inherited thrombocytopenias have characteristic features: giant platelets in May-Hegglin anomaly, Alport’s syndrome variants (associated with renal insufficiency and hearing loss) and Bernard-Soulier syndrome; small platelets in Wiskott-Aldrich syndrome; skeletal or growth abnormalities in the thrombocytopenia with absent radius syndrome and Fanconi’s anemia. The giant platelet syndromes may be confused with chronic ITP because platelets are often larger than normal in ITP. However, the occurrence of many, truly giant platelets (approaching the size of erythrocytes) is evidence against the diagnosis of ITP.

Patients with familial thrombocytopenia presenting without defining features are probably more common than those with these recognizable syndromes. Inheritance patterns in these families are most commonly autosomal dominant, though autosomal recessive and X-linked recessive patterns also occur. Some patients with mutations in the WASP gene have only X-linked thrombocytopenia with small platelets and no other clinical manifestations of the Wiskott-Aldrich syndrome.

DISORDERS OF PLATELET FUNCTION

Most platelet function disorders are resulting from hereditary abnormality resulting from abnormalities of membrane proteins, granule constituents, or metabolic reactions are not clinically severe and clearly defined. Common among these are disorders of the dense granules with diminished secretion of ADP, termed “storage pool disease.” This abnormality may be associated with oculocutaneous albinism, the Hermansky-Pudlak syndrome. Two other disorders, Glanzmann’s thrombasthenia and Bernard-Soulier syndrome, are both recognizable from clinical evaluation and understandable from a molecular perspective.

Classification

Disorders of platelet function may be classified as below:
1. Hereditary disorders of platelet function
   a. Adhesion defect
      Bernard-Soulier syndrome
b. Aggregation defect
   Glanzmann’s thrombasthenia

c. Secretion disorders
   i. Granule deficiencies
      — α granule abnormalities
      — Gray platelet syndrome
   ii. δ granule dense body abnormalities
      — Storage pool disease; isolated dense body deficiency
      — Hermansky-Pudlak syndrome
      — Chediak-Higashi syndrome
      — Wiscott-Aldrich syndrome
      — Thrombocytopenia and absent radii
      — α/δ granule deficiency
   iii. Defects of signal transduction and secretion
      — Impaired liberation of arachidonic acid
      — Cyclo-oxygenase deficiency
      — Thromboxane synthetase deficiency
      — Thromboxane A₂ receptor abnormalities
      — Defective Ca⁺ mobilization
   iv. Defects of platelet coagulant activity

v. Miscellaneous
   — Hereditary macrothrombopathy/sensorineural hearing loss

2. Acquired disorders of platelet function
   a. Drug-induced platelet dysfunction
      i. Analgesic
      ii. Antibiotics
      iii. Cardiovascular drugs
      iv. Psychotropic drugs
   b. Uremia
   c. Disorders of hemopoietic system
      i. Paraproteinemias
      ii. MDS/acute nonlymphocytic leukemia
      iii. Myeloproliferative disorders.

Glanzmann’s Thrombasthenia

Glanzmann’s thrombasthenia is an autosomal recessive disorder caused by an abnormality of GP IIb-IIIa, resulting in absent or defective fibrinogen binding and absent platelet aggregation to all physiologic agonists. Because the gene frequency is rare, most cases of Glanzmann’s thrombasthenia occur in families with consanguinity. Mucocutaneous bleeding, similar to patients with ITP and von Willebrand’s disease (vWD), includes purpura, epistaxis, gingival bleeding, and menorrhagia. In spite of lifelong absence of platelet aggregation, major bleeding in the absence of trauma (except for menorrhagia and postpartum hemorrhage) is rare. These clinical observations provided part of the rationale for safety of antithrombotic agents that block GP IIb-IIIa function. Acute bleeding can be effectively treated with platelet transfusions; persistent mucosal bleeding may be controlled with ε-aminocaproic acid.

Bernard-Soulier Syndrome

Bernard-Soulier syndrome is caused by an abnormality of GP Ib-IX-V, the receptor for vWF. The inheritance, frequency of consanguinity, and clinical manifestations are similar to Glanzmann’s thrombasthenia. Distinctive features of Bernard-Soulier syndrome are giant platelets and thrombocytopenia; this suggests a role for GP Ib-IX-V in megakaryocyte development. Laboratory diagnosis is characterized by normal platelet aggregation to physiologic agonists but defective agglutination of patient platelet-rich plasma in response to ristocetin.

Acquired Disorders

In contrast to the congenital disorders of platelet function, acquired abnormalities are very common. These disorders may be classified as under.

Acquired abnormalities of platelet function due to:

1. Drugs, foods, spices
2. Systemic conditions
   a. Chronic renal disease
   b. Cardiopulmonary bypass
3. Hematologic disorders
   a. Antiplatelet antibodies
   b. Myeloproliferative and lymphoproliferative disorders
   c. Dysproteinemias.

Drugs, Foods, and Spices

Over 100 drugs, foods, and spices have been shown to affect platelet function. The clinical importance of these observations is unknown. Almost all reports describe impairment of in vitro platelet aggregation, and some reports have described an association with a prolonged bleeding time; however, a causal relation to clinically important bleeding is rarely documented. An exception is aspirin, but even for aspirin reports in otherwise normal subjects are conflicting. Ticlopidine and clopidogrel are more potent inhibitors of
platelet function than aspirin, but there is no evidence that these agents cause more bleeding than aspirin. Drugs that inhibit the function of platelet GP IIb-IIIa, creating an acquired form of Glanzmann’s thrombasthenia, are associated with an increased risk for bleeding, but they are typically used in combination with heparin and aspirin.

**Systemic Conditions**

Platelets from patients with chronic renal failure have defects in adhesion, aggregation, secretion, and procoagulant activity, and the bleeding time may also be prolonged. However, dialysis and nutritional support have improved the management of patients with chronic renal failure so that bleeding is no longer an important issue. Diminished bleeding complications may also result from use of erythropoietin to correct anemia.

The clinical importance of other acquired disorders, such as myeloproliferative disorders, myeloma, and macroglobulinemia, cardiopulmonary bypass surgery, and the presence of antiplatelet antibodies, is unclear. Abnormal in vitro platelet function can be shown, but the relation of these abnormalities to bleeding is unknown.

**VON WILLEBRAND’S DISEASE**

Two major roles in hemostasis are performed by vWF: it mediates the adhesion of platelets to sites of vascular injury via vWF’s interaction with platelet GP Ib and the subendothelial matrix, and it is a carrier protein for factor VIII. Defects in vWF, therefore, may cause bleeding by impairing both platelet adhesion and blood clotting. vWD is caused by an inherited mutation in the vWF gene found on chromosome 12. The disorder typically demonstrates autosomal dominant inheritance, although several subtypes are inherited in a recessive fashion. The overall prevalence of vWD is approximately 1 percent in the population with no differences across racial or ethnic groups. The classification for vWD includes three major categories: partial quantitative deficiency (type 1), qualitative deficiency (type 2), and total deficiency (type 3). Qualitative deficiencies (type 2 vWD) are further divided further into four variants or subtypes (2A, 2B, 2M, and 2N) based upon the nature of the phenotype. These six categories correspond to distinct pathophysiologic mechanisms, some with unique therapeutic requirements (Table 6.4).

**Testing for vWD**

Laboratory studies are directed at documenting vWF deficiency or qualitative vWF defects.

Screening tests for vWD include the APTT, ristocetin cofactor activity (vWF:RCo), and vWF antigen (vWF:Ag). Antigen levels are determined by either the Laurell rocket electrophoretic methods or, more commonly, by enzyme-linked immunosorbent assay (ELISA). Factor VIII levels are done concomitantly as they typically mirror the decrement in antigenic vWF levels. In a patient being evaluated for abnormal bleeding, a disproportionately low factor VIII level compared to vWF:Ag suggests the presence of hemophilia A, a defect in vWF that impairs the binding of factor VIII to vWF (vWD type 2N); or, in adults, another cause of factor VIII deficiency such as an antifactor VIII antibody. The ristocetin cofactor activity measures the functional activity of vWF. vWF:RCo is performed by adding ristocetin, an

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<th>Table 6.4: Classification of von Willebrand’s disease</th>
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<td><strong>Type 1</strong></td>
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antibiotic that induces platelet agglutination via the vWF-GP Ib/IX/V interaction, to the patient’s platelet poor plasma and fixed normal platelets. Multimer analysis evaluates the size of the multimeric vWF complex when separated on an agarose gel via electrophoresis. Further tests may be necessary to subtype the patient’s specific defect. These additional tests include ristocetin-induced platelet aggregation (RIPA), factor VIII binding assay (ELISA), and DNA sequencing of the gene. The platelet function analyzer (PFA-100) is being established as a sensitive screening tool for vWD, and the GP Ib binding and collagen binding assays (ELISA) are being evaluated for their measurement of vWF function. The template bleeding time is not recommended as a screening test for vWD due to its relative lack of sensitivity for type 1 disease, the most common form of vWD.

vWF levels vary with physiologic stress and estrogen. Therefore, testing should not be done when the patient is experiencing an acute hemorrhage or infection, during periods of strenuous exercise or emotional stress, and during pregnancy or use of hormonal therapy. Other causes of secondary (nongenetic) low vWF levels include Wilms’ tumor, ventricular septal defects and other congenital heart diseases.

Evaluation of vWF:RCo and vWF:Ag is further complicated by the broad range of normal values for plasma vWF concentration and by the pronounced dependence of plasma vWF levels on ABO blood type. On average, the mean vWF:Ag level for healthy persons with blood type 0 is approximately 25 percent lower than that of persons with other blood types, and these values overlap with those from patients with type 1 vWD. Commonly, the vWF:RCo lower limit of normal for unaffected individuals with blood types A, B, and AB is 55 percent compared to 35 percent for those unaffected individuals with blood type O. Determination of the normal range in people with blood type 0 and non-O should be established at each laboratory that performs vWD testing.

**Type 1 vWD**

Type 1 vWD accounts for more than 70 percent of patients with vWD and is a genetic disease of mucocutaneous bleeding caused by partial, quantitative vWF deficiency. Unlike patients with type 2 disease, many patients with type 1 vWD do not have a defined gene mutation. The most common symptoms are epistaxis, skin bruises and hematomas, prolonged bleeding from trivial wounds and postpartum, oral cavity bleeding, and menorrhagia. Many patients will have only mild symptoms and inconsistent histories of abnormal bleeding. Neonates with type 1 disease rarely bleed and establishment of the diagnosis at this time is difficult, in part due to high levels of vWF in the fetus and newborn. In patients with type 1 vWD, testing typically reveals a low vWF:Ag proportional to a low factor VIII level and vWF:RCo. Multimer analysis reveals the presence of all forms but in diminished amounts.

**Type 2 vWD**

Qualitative dysfunction of the vWF protein assembly is the hallmark of the type 2 vWD defects. vWF can mediate platelet adhesion normally only if it is assembled into large multimers, and stabilization of factor VIII requires a functional factor VIII binding site on vWF. Mutations in type 2 vWD can disrupt any of these properties. Such qualitative abnormalities of vWF often are signaled by discrepancies among the various assays of vWF concentration and function. The type 2 mutations cluster in regions of the gene that impact the protein’s function.

**Type 2A vWD**

The hemostatically active large multimer forms of vWF are deficient in patients with subtypes 2A and 2B vWD. On laboratory testing, a disproportionately low vWF:RCo relative to vWF:Ag reflects the decreased affinity of vWF for platelets. The most common cause of such loss of function is the absence of large vWF multimers characteristic of type 2A vWD. Type 2A disease usually is transmitted as a dominant disorder, causing the absence of large and intermediate-sized vWF multimer forms either by impairing multimer assembly within the cell or by promoting the clearance of large multimers from the circulation, possibly by proteolysis. One mechanism for type 2A vWD is mutations that increase susceptibility of vWF for proteolytic cleavage in plasma by the vWF-cleaving protease, ADAMTS13 (the protease which is deficient in congenital and some acquired TTP-HUS syndromes).
Type 2B vWD

Type 2B vWD is characterized by increased affinity of the mutant vWF for platelets, causing spontaneous binding of large vWF multimers to platelet receptor GP Ib, followed by clearance of both vWF and platelets. The remaining smaller multimers are not hemostatically effective, and the patients have a bleeding diathesis that may seem severe relative to the degree of vWF deficiency. Thrombocytopenia in type 2B disease may be intermittent and often is exacerbated by stress, infection, or pregnancy. Type 2B vWD may be initially misdiagnosed as ITP. Although vWF levels occasionally are in the normal range, the vWF:Ag usually is decreased. The diagnosis of type 2B vWD depends on the results of RIP A in the patient’s platelet-rich plasma. In type 2B disease, brisk platelet aggregation occurs at a low concentration of ristocetin (0.5 mg/ml) that has little or no effect on platelet-rich plasma from normal control subjects. Positive results of this test are found in only one other rare disease, platelet-type or pseudo-vWD, in which mutations in platelet GP Ib cause a phenotype very similar to that of type 2B vWD.

Type 2M vWD

vWD type 2M (“M” for “multimer”) disease includes variants in which binding to platelets is impaired, but the vWF multimer distribution is normal. This rare type of vWD phenotype may be produced by mutations that inactivate specific binding sites for platelets or connective tissue. Laboratory results reveal a disproportionate decrease in vWF:RCo and a normal multimer pattern.

Type 2N vWD

Mutations that selectively inactivate the factor VIII binding site on vWF produce an autosomal recessive vWD phenotype in which the platelet-dependent functions of vWF are preserved, but factor VIII levels are low, often less than 10 percent. This variant is type 2 Normandy (type 2N) vWD. These patients may have been misdiagnosed with hemophilia A, although the inheritance pattern suggests an autosomal trait. Suspected hemophilia A carriers with unusually low factor VIII levels should be screened for this abnormality. Screening test results typically show normal vWF levels and a prolonged APTT. The definitive diagnosis requires demonstration that the patient’s vWF has decreased affinity for factor VIII or by molecular studies of the vWF gene. The diagnosis of type 2N vWD should be considered in any patient with a low factor VIII level in whom: (i) a factor VIII inhibitor is ruled out; (ii) the evidence for X-linked inheritance is not clear; and (iii) initial therapy with factor VIII concentrates (not containing vWF) gives unexpectedly poor results.

Type 3 vWD

Type 3 vWD is a recessive disorder in which there is virtually no detectable vWF. This causes a secondary marked deficiency of factor VIII, and afflicted patients have a combined defect in platelet adhesion and blood clotting. Screening assays show absent vWF:RCo and vWF:Ag, as well as a prolonged APTT explained by a low factor VIII level. The parents of patients with type 3 vWD may have bleeding symptoms and meet criteria for type 1 disease, but most appear to be normal.

Treatment of vWD

Desmopressin [l-desamino-8-D-arginine vasopressin (DDAVP)] is the treatment of choice for patients with type 1 vWD. DDAVP is a synthetic analog of the antidiuretic hormone vasopressin with enhanced antidiuretic activity and little pressor activity. The infusion of DDAVP into healthy persons and patients with type 1 vWD results in a rapid increase in circulating levels of vWF:Ag, vWF:RCo, and factor VIII. The increase in VWF is presumably due to release of stored vWF from Weibel-Palade bodies in the vascular endothelium, a tissue rich in vasopressin membrane receptors. The optimal hemostatic effect is produced by 0.3 μg/kg of DDAVP (maximal dose, approximately 25 μg) infused intravenously over 30 minutes. The typical maximal increase is two-fold to four-fold for vWF and three-fold to six-fold for factor VIII, and hemostatic levels of both factors are usually maintained for at least 6 hours. Repeated doses of DDAVP have been reported to result in tachyphylaxis; therefore, repeat infusions are generally delayed for 24 to 48 hours. Facial flushing is a common side effect. Blood pressure and serum sodium should be monitored, and patients should be fluid restricted to avoid rapid development of hyponatremia and
seizures. This complication is most frequent in infants and young children, elderly individuals, and ill patients receiving intravenous fluids. Intranasal administration of a concentrated intranasal formulation is often useful for home therapy of mild bleeding complications or menorrhagia.

Many patients with types 2A and 2M disease will have a favorable response to DDAVP, but the duration of response is often shorter than in patients with type 1 vWD. For prophylaxis for major surgery or for the treatment of serious bleeding episodes, vWF-containing factor VIII concentrates are the treatment of choice.

In patients with type 2B vWD, DDAVP releases the abnormal vWF from endothelial cells. Circulating platelet aggregates and worsening of thrombocytopenia have been reported in patients with type 2B vWD after DDAVP administration. For serious bleeding and major surgical procedures, vWF-containing factor VIII concentrates should be administered.

In patients with type 2N vWD, infusion of factor VIII concentrates that do not contain significant amounts of vWF, such as monoclonally purified or recombinant factor VIII concentrates, will lead to only a transient rise in factor VIII levels, with a half-life of approximately 2.5 hours. It is thus important that the correct diagnosis be made so that vWF-containing factor VIII concentrates are used. The use of DDAVP therapy in type 2N disease may cause only a transient increase in factor VIII levels because the released vWF is unable to bind to, and stabilize, factor VIII. Patients with an inadequate response to DDAVP and those patients with type 3 vWD must be treated with vWF-containing factor VIII concentrates.

The vWF-containing factor VIII concentrates are virally inactivated. A purified vWF concentrate is used in Europe, although it is not available in the USA, and no recombinant product is available to date. Transfusion with fresh frozen plasma (FFP) or cryoprecipitate should not be considered because these products do not contain enough vWF (particularly FFP) and cannot be virally inactivated (cryoprecipitate).

**Pseudo-vWD**

Pseudo-vWD (platelet-type vWD), a rare platelet disorder mimicking type 2B vWD, is caused by a congenital abnormality of GP Ib-IX-V resulting in increased affinity for large vWF multimers. Management of bleeding requires platelet transfusion.

**Acquired vWD**

vWD caused by increased clearance of plasma vWF is a rare acquired bleeding disorder. The clinical manifestations are those of vWD, except for the adult onset. The disease is virtually unheard of in children. The laboratory diagnostic features are similar to those of type 2B vWD with depletion of the large multimers, and tests for vWF:Ag may be normal. Most patients have an underlying lymphoproliferative disorder or monoclonal gammopathy with antibody activity against vWF. In other patients, vWF is depleted by clearance onto vWF receptors, as in essential thrombocytosis or tumors expressing vWF receptor activity. Replacement therapy may be less effective than in inherited vWD, especially in patients with neutralizing antibodies. Sustained response require control of the underlying disease.

**PLATELET TRANSFUSION THERAPY**

In the practice of transfusion medicine, it is often required and mandatory to use platelet products for the optimal care of the critically ill patients. Today with the advent of component therapy and availability of cell separators, it is possible to individualize the transfusion therapy in most of the cases. Platelet preparations available include the following.

**Platelet Concentrates and Apheresis Platelets**

Platelet transfusions are available as platelet concentrates or as apheresis units. The former are prepared from units of whole blood by centrifugation and the latter are collected by pheresis devices. A variety of scientific arguments has been proposed for the superiority of apheresis platelets, including reduced rates of alloimmunization and transfusion reactions. However, the only compelling reason seems to be the reduced infectious risk with apheresis platelets. In the USA, platelet concentrates are separated from whole blood by first preparing platelet-rich plasma and then centrifuging the platelets with a second centrifugation. The contents of the platelet concentrates are highly variable depending upon technique. However, the 50 ml platelet concentrate usually has at least $5.5 \times 10^{10}$ platelets. In Europe, the buffy coat method of pre-
paration is the most common method of platelet preparation. This method involves centrifugation to prepare a buffy coat, from which platelets are separated by an additional centrifugation. The white cell contamination is approximately $10^8$ per bag using the platelet-rich plasma method of concentration, and $10^6$ for platelets prepared by the buffy coat method. The relatively lower white blood cell (WBC) content in platelets prepared by the buffy coat method may be advantageous in reducing alloimmunization and febrile transfusion reactions.

Pheresis platelets are collected from donors by continuous centrifugation using a large intravenous catheter which allows processing of large volume of blood and the removal of platelets using an automated system. Since a conventional transfusion dose for an adult patient is approximately 6 U of pooled platelets, collection parameters have been used to collect this number of platelets from a donor. Modern pheresis devices are equipped to predict the yield from the donor’s size, platelet count and hematocrit. The leukocyte content of a pheresis platelet unit depends upon the technology used, but most devices have WBC contamination of less than 106 per bag. Single donors pose less infectious risk to the recipient than do pooled platelets simply because there are fewer donor exposures. For several years, there has been a great deal written about the benefits of pheresis platelets in reducing the risk of alloimmunization. Data from the TRAP trial have shown that leukocyte-reduced pheresis platelets provide no additional reduction in alloimmunization compared to leukocyte-reduced platelet concentrates.

Platelet blood components are licensed for storage time of 5 days at room temperature. Clinical studies indicate that there is little loss of platelet function and viability with 5-day-old platelets. Maintenance of the function and viability of liquid-stored platelets is limited to a relatively short period of time because of the storage lesion that develops. There has been a great deal of interest in improving the storage condition for platelets to reduce the functional abnormalities that occur during storage. When platelets are removed from the circulation and exposed to the foreign conditions of the most carefully designed collection and storage system, a variety of changes collectively referred to as platelet activation begin. A number of variables may produce minimal changes that perpetuate the process as more platelets are recruited into the activated state. These activation changes may be reflected in platelet shape change, adhesion, aggregation, secretion of platelet granular contents, and the expression of activation antigens. The challenge of preparing platelets for transfusion has been to minimize the damaging effects of preparation and storage.

Cryopreserved Platelets

Cryopreserved platelets have been developed for long-term platelet storage using dimethylsulfoxide or glycerol. Extensive research has been done on both of these agents. The average post-transfusion recovery of cryopreserved platelets is approximately 50 percent. Hemostasis seems to be maintained with dimethylsulfoxide (DMSO) preserved platelets. Despite early promising results from glycerol-preserved platelets, this has not been successfully adapted for clinical practice. Cyropreserved platelets were especially developed for patients who become alloimmunized during induction chemotherapy for acute leukemia and later required additional marrow suppressive therapy. Platelets could be collected during remission, frozen and subsequently used when necessary. Since using frozen platelets presents some logistical considerations, they are not widely used.

Lyophilized Platelets

The lyophilization of platelets is an alternative that circumvents these logistical concerns inherent to frozen-stored platelets. Recently, Read and coworkers reported that lyophilized platelets may support hemostasis in an animal model. They were able to show that they could correct the bleeding time in thrombocytopenic animals and also that the transfusion of reconstituted lyophilized platelets participated in carotid arterial thrombus formation in a canine model. This form of platelets is currently undergoing human clinical trials and is not yet approved for routine use. Although these studies are provocative, additional studies are needed to substantiate the usefulness and safety of lyophilized platelets and to investigate potential other forms of platelet substitutes.
Plasma Products
Cryoprecipitate is prepared by thawing fresh-frozen plasma at 4°C and then removing the supernatant from the cryoprecipitable proteins by centrifugation at 1 to 6°C. Use of the supernatant plasma has been explored in the treatment of thrombotic thrombocytopenic purpura (TTP), because it lacks the high-molecular-weight multimers of vWF that may be involved in the pathogenesis of TTP. Cryoprecipitate’s main use is for fibrinogen replacement and for treating uremic platelet dysfunction.

New viral inactivation methods exist to sterilize plasma. The most commonly employed technique to treat plasma uses detergents that disrupt lipid-containing viruses. It has been shown that a five-log reduction of virus is achievable in plasma derivatives such as factor VIII, antithrombin III concentrate, and prothrombin concentrates. In these treatments functional recovery of clotting factors is well preserved. Recently available is a solvent-treated form of FFP. Solvent detergent treatment of plasma and plasma fractions has virtually eliminated the risk of hepatitis B and C and retrovirus transmission in patients receiving FFP.

Platelet Transfusions
Platelet transfusions are given prophylactically or therapeutically in thrombocytopenic patients and for the performance of invasive procedures. There has been considerable interest in trying to define the lowest safe platelet concentration at which bleeding is unlikely and so that fewer prophylactic transfusions would be given. This interest was initially stimulated by the hypothesis that if fewer platelets were given, alloimmunization rates would be lower. This, however, does not appear true in acute leukemic patients. There does not appear to be a dose-response to the number of platelet concentrates given and the rate of alloimmunization. Certainly, by lowering the level at which one transfuses platelets, the expense of platelet transfusions may be decreased. Prior to the advent of effective platelet transfusion support, hemorrhage accounted for more than 50 percent of the deaths in acute leukemia. With the advent of effective platelet transfusion support, death due to hemorrhage was reduced to less than 5 percent. In an effort to identify a “hemorrhage threshold” related to the degree of thrombocytopenia, Gaydos et al reviewed hemorrhagic episodes in 92 consecutive patients treated for acute between 1956 and 1959 at the National Cancer Institute. Platelet counts below 100,000/mm³ were associated with an increased risk of bleeding. Patients with platelet counts of 5,000/mm³ or less manifested gross hemorrhage on approximately one-third of days at risk. The authors, however, could not identify a distinct threshold. Rather, the risk of hemorrhage increased progressively as the platelet count fell. In examining patients with fatal hemorrhage, these authors also noted that patients in “blast crisis” manifested hemorrhage despite adequate platelet counts. Excluding such patients, only eight of the 92 cases developed fatal hemorrhage. Of these eight cases, only one patient with fatal hemorrhage had a platelet count >5,000/mm³, and none exceeded 10,000/mm³.

In the 1960s and 1970s, platelet concentrates were not available on an emergency basis. Various methodologies of storing platelets, some clearly less than optimal, were employed resulting in platelet concentrates of variable efficacy. Further, there was a general clinical feeling that hemorrhagic episodes were catastrophic and irreversible. In this context, a prophylactic platelet transfusion strategy was generally accepted and a 20,000/mm³ “trigger” was adopted.

More recently, in a randomized study of prophylactic platelet transfusion threshold during induction therapy in adult patients with acute leukemia, Heckman and coworkers showed that giving prophylactic transfusions only when the platelet count dipped below 10,000/mm³ decreased platelet utilization with only a small adverse effect on bleeding and no effect on mortality. It, therefore, appears that with amegakaryocytic thrombocytopenia, prophylactic transfusions should be given if the count falls below 5,000/mm³. At values between 5,000 and 10,000/mm³, one may be able to abstain from transfusing if the patient is stable and if no other conditions make spontaneous bleeding likely. These conditions include blast crisis, anticoagulation with heparin for disseminated intravascular coagulation, drugs that affect platelet function, uremia, and recent invasive procedures, including spinal taps or the placement of central venous catheters.
Platelet Transfusion Dose

The dose that one administers to a thrombocytopenic patient is dependent upon the therapeutic goal. If one is administering prophylactic platelet transfusions in a myelosuppressed patient, one may only wish to administer sufficient platelets so as to prevent bleeding. In this situation, the general practice is to administer sufficient platelets to maintain a sustained platelet count above 10,000 to 20,000/mm³. To accomplish this, one must take into consideration different physiologic considerations, including the presence of fever, active bleeding, and disseminated intravascular coagulation. Approximately one-third of transfused platelets are sequestered in the splenic pool. Because of this, approximately one-third more platelets need to be transfused to accomplish a target platelet count. Although normal platelet survival is about 9 days, there exists a direct relationship between the platelet count and survival of platelets in thrombocytopenic individuals. The survival of transfused platelets may be followed by frequent platelet counts to estimate the frequency of transfusion.

Knowing the concentration, or average concentration of platelets supplied by a blood center, one can calculate the volume of platelets to transfuse. The Food and Drug Administration (FDA) guidelines dictate that pheresis platelets must contain >3 × 10¹¹ platelets (six equivalent units), or >5.5 × 10¹⁰ in platelet concentrates prepared from units of whole blood. In a normal sized individual, approximately 3 × 10¹¹ platelets is considered an appropriate dose.

EVALUATION OF PLATELETS

Quantitation of Platelets

Platelet numbers are quantitated accurately by automated cell counters that depend on electrical impedance or light scatter. Other methods for quantitating platelets and potential artifacts that lead to falsely low platelet counts are described above, automated cell counting, platelet analysis.

Platelet Function Tests

The most important evaluation of platelets is their number; peripheral blood film examination confirms the platelet count and provides information about platelet morphology. Giant platelets are seen in Bernard-Soulier syndrome and other congenital disorders, whereas small platelets are seen in patients with Wiskott-Aldrich syndrome.

Detailed Evaluation of Platelet Function

Platelet Aggregation

Platelet aggregation, a very sensitive and time-consuming test, has been the primary method available for evaluation of platelet function until recently. In this test, either whole blood or platelet-rich plasma is prepared, and agonists such as adenosine diphosphate (ADP), epinephrine, collagen, arachidonic acid, or thrombin are added separately to a stirred aggregometer tube to initiate platelet aggregation. The formation of platelet aggregates causes an increase in light transmission through the PRP as the aggregates fall to the bottom of the tube. The change in light transmission is recorded by a mechanized recording instrument. The platelet release reaction can be assessed during the assay by the addition of a reagent that requires adenosine triphosphate (ATP) for signal production. Although measurement of platelet aggregation can be very helpful, its time-consuming nature, the need for a fresh patient sample, the technical skill demanded in its performance, and the lack of quantitative parameters for its interpretation have decreased its use.

Platelet Function Analyzers

Platelet function analyzers (PFAs) have been developed as less complicated methods to measure platelet plug formation in vitro. One instrument, the platelet function analyzer (PFA-100), has been shown to be a more sensitive screening tool than the bleeding time for patients suspected of having vWD or a primary platelet defect. However, the specificity of this screening tool is no better than the bleeding time and the use of the PFA-100 in predicting surgical bleeding has not been validated.

PFA-100 Citrated blood is exposed to epinephrine or ADP as it is aspirated at arterial shear rates to a membrane coated with collagen that contains an aperture. The platelets are activated, aggregate, and
form a plug that occludes the aperture in the membrane; the endpoint is measured as the time required for a drop in pressure within the system caused by the occlusion of the aperture. The instrument is sensitive to intrinsic platelet function as well as to vWF levels. Though not as sensitive or specific as platelet aggregation studies, it is technically easy to perform, is more quantitative and reproducible, and is much less time consuming.

Template Bleeding Time

The template bleeding time is used for the diagnosis of intrinsic platelet abnormalities, and it is also abnormal in moderate and severe vWD. The test is performed by making a standard incision in the forearm using controlled conditions and measuring the time for the bleeding to stop. It is operator dependent, it reflects the integrity of the microvasculature as well as the platelets, and it is not particularly sensitive. Most importantly, it does not reliably predict the individual’s hemostatic capacity and should not be used as a general preoperative screening study.

Assays for Platelet Antibodies

Assays for platelet antibodies may be performed as part of an evaluation for immunologic causes of thrombocytopenia. Flow cytometry has been used to measure the immunoglobulin on the surface of platelets, but this test does not reflect specific platelet antibodies and has not correlated well with the clinical status of patients with immune thrombocytopenias. More specific tests, using specific monoclonal antibodies to platelet antigens such as GP Ib and IIb/IIIa, can be performed in microtiter plate assays using a monoclonal antibody immobilization of platelet antigen (MAIP A) format. These tests require sufficient numbers of patient platelets to provide the platelet-bound antibody, and sufficient platelets are not always available from patients with ITP. The tests are positive in only 50 to 70 percent of patients with clinically diagnosed ITP. A technically similar indirect test using normal platelets exposed to patient serum is positive in only 30 to 50 percent of patients. These tests are difficult to perform, but may be helpful when they are positive and are sometimes used for following the patient through courses of treatment.

Assays for Heparin-Induced Thrombocytopenia

Assays for heparin-induced thrombocytopenia (HIT) can be performed by several different methods. One of the most widely used tests is an ELISA assay that measures antibodies against platelet factor 4-heparin complexes. These are sensitive assays that detect antibodies that may not be clinically significant as well as antibodies that cause thrombocytopenia and thrombosis. They must be interpreted within the clinical context. A functional assay, deemed the gold standard by many hematologists, is the serotonin-release assay: donor platelets are incubated with radiolabeled serotonin, the platelets internalize the serotonin, and they are subsequently exposed to the patient’s serum and heparin at a concentration of 0.1 to 0.2 U/ml. The platelets undergo a release reaction if antibodies to the PF-4-heparin complex are present, and the released radiolabeled serotonin is measured. A control incubation is performed using a high concentration of heparin (~10.0 U/ml) to ensure specificity (the high concentration of heparin prevents the correct stoichiometric complex of PF-4 and heparin, and no release reaction occurs). Another functional test, the heparin-induced platelet activation test, is used more widely in Europe; it is less specific than the serotonin release assay, but it does not require the use of radioactivity. Clinically, it is important to discontinue heparin immediately and institute another anticoagulant if heparin is thought to be a possible cause for thrombocytopenia or thrombosis in a patient; there can be both false-positive and false-negative tests for HIT.

SELECTED FURTHER READING

5. Fitzgerald LA, Phillips DR. Platelet membrane glycoproteins. In Colman RW, Hirsh J, Marder VJ, Salzman EW,


ABSTRACT
Blood platelets play a very important role in maintaining normal hemostasis. They have broad spectrum of activities and modulate both physiological functions and physiological disorders. Their role in hemostasis in response to vascular injury is well documented. Following vascular injury, the platelet-related events that occur include: adhesion, aggregation, secretion of granule contents, initiation of clot formation, promotion of clot retraction and modulation of wound healing. No single in vitro test has stood out as a gold standard for documenting the platelet function and their efficacy. Currently, it has been recognized that a battery of tests, which examine different aspects of platelet physiology, can provide a reasonable assessment of platelet efficacy in vivo. Current gold standard for platelet performance is in vivo survival of transfused radiolabeled platelets. It is based mostly on the assumption, that viable platelets in circulation will meet their obligation and participate in the expected physiological responses. However, not much of clinical data are available to support this assumption. General consensus at present in the research community is that all the novel procedures available on platelet function testing should be evaluated against accepted conventional methods currently being used in the blood banking practice. In this chapter, an attempt has been made to provide information on a useful preservation method and a simple platelet function testing technique.

INTRODUCTION
Human blood has been used successfully for transfusion therapy for several decades. Platelet concentrates (PC) prepared by the blood procurement centers are used primarily for the treatment or prevention of bleeding. These cells, stored as concentrates, lose functional capability during their storage period. Past research has focused on improving the procurement of blood, formulating the ideal preservation solution, improving the processing of concentrates, designing the ideal type of storage bag, and improving the environment in which platelets are stored.

The collection process, the storage conditions, and the duration of storage collectively lead to inducement of storage lesions. Such lesions might be mediated by alterations in the biochemical or metabolic activity of the stored platelets, leading to an ultimate compromise in in vitro function. For instance, irreversible changes in the shape of platelets, elevation of cytosolic calcium, assembly of filamentous actin, loss of activity of enzymes that play a critical role in signal transduction events, loss or decrease in available receptors, or loss of adenylate energy charge are considered storage-induced lesions. These lesions singly or together will be reflected in a platelet’s viability and function. Therefore, there is a great immediate need for developing an appropriate preservative to improve the function of stored platelets and a simple device that can assess the functionality of these preserved
cells prior to transfusion. In this chapter, we provide the readers an overview on the recent advances made in the area of platelet preservation and the development of platelet function testing devices.

Since the middle of 1980s, there have been many studies in which the goal was to improve the quality of PC stored for 5 days or to possibly extend storage time. Unfortunately, none of those attempts have been successful enough to become a standard procedure in the processing, preparing and storing of PC. Five-day shelf-life for stored platelets has been regulated in view of their rapid loss of in vitro function during storage and the increase in bacterial contamination that may occur with extended storage. Because of this quandary, a new, proprietary experimental preservative solution, ViaCyte™, was developed and evaluated by assessing its effect on functional parameters of stored platelets. The results of this study using this experimental solution as a preservative demonstrates its protective properties on stored platelets.

**PLATELET BIOCHEMISTRY AND FUNCTION**

Blood platelets interact with a variety of soluble agonists such as epinephrine and adenosine diphosphate, many insoluble cell matrix components, including collagen, laminin, and biomaterials used for the construction of invasive medical devices. These interactions stimulate specific receptors and glycoprotein-rich domains (integrins and non-integrins) on the plasma membrane and lead to the activation of intracellular effector enzymes. The majority of the regulatory events appear to require free calcium. Ionized calcium is the primary bioregulator, and a variety of biochemical mechanisms modulate the level and availability of free cytosolic calcium. Major enzymes that regulate the free calcium levels via second messengers include phospholipase C, phospholipase A₂, and phospholipase D, together with adenylyl and guanylyl cyclases. Activation of phospholipase C results in the hydrolysis of phosphatidylinositol 4, 5-bisphosphate and formation of second messengers 1, 2–diacylglycerol and inositol 4, 5-bisphosphate (IP₃). Diglyceride induces activation of protein kinase C, whereas IP₃ mobilizes calcium from internal membrane stores. Elevation of cytosolic calcium stimulates phospholipase A₂ and liberates arachidonic acid. Free arachidonic acid is transformed to a novel metabolite, thromboxane A₂ by fatty acid synthetase (COX-1, cyclo-oxygenase). Thromboxane A₂ is the major metabolite of this pathway and plays a critical role in platelet recruitment, granule mobilization and secretion. Secretory granules contain a variety of growth factors, mitogens and inflammatory mediators. Secretion of granules promotes P-selectin expression on the platelet membrane. Furthermore, activation also promotes the expression of acidic lipids on the membrane and tissue factor expression, thus making these cells procoagulant. Fully activated platelets can modulate the function of other circulating blood cells such as leukocytes, monocytes, and macrophages as well as vascular endothelial cells. Although platelets that are not activated, bind surface-bound fibrinogen, they cannot bind soluble fibrinogen. Agonist-mediated stimulation of platelets promotes the expression of an epitope on glycoprotein (GP) Ib-IIIa receptors. Activation of this receptor is essential for the binding of circulating fibrinogen. Fibrinogen forms a bridge between individual platelets and facilitates the thrombus formation. von Willebrand Factor (vWF) binds platelet GP Ib-IX complex only at high shear rate unlike fibrinogen, which can bind platelets at low shear. Upregulation in signaling pathways will increase the risk for clinical complications associated with acute coronary events. Downregulation of signal transduction mechanisms may precipitate bleeding diathesis or stroke.

**In Vitro Evaluation of Platelet Biochemistry and Function**

Platelet morphology should be visually inspected at different phases of storage to identify the percentage of cells that are discs vs spheres. In some blood banks, there are automated devices capable of screening the bags to determine the change in the cell morphology. Monitoring levels of ATP, glucose and lactate should assess biochemical status of these cells. For instance, drop in platelet count and increase in the lactate will suggest cell lysis. The pH of platelet suspension above 7.6 and below 6.2 has been shown to correlate with poor in vivo performance. Markers to determine the activation of platelets during processing of blood include: surface expression of granule membrane protein (GMP-140, P-selectin, CD 62), CD63 and active epitope on GPIIb-IIIa, which binds fibrinogen (PAC-1). In addition, thromboglobulin, platelet factor-4
(PF-4) and other granule-related proteins could be measured as index of activation of platelets. Functional capability of platelets can be assessed by their response to osmotic stress, the extent of agonist-induced activation, aggregation in response to increasing concentrations of single agonists, dual agonist combinations (ADP/Epinephrine or ADP/collagen). Ability of stored platelets to secrete granule contents can be monitored by measuring P-selectin or released ATP. In addition, the presence of microparticles can be monitored by flow cytometry as it will help characterize the product better. All these tests should be run as paired comparison with platelets stored in the FDA-approved storage containers. When testing a new preservative, the in vitro test conditions should mimic the exact conditions encountered by the platelets.

**FDA RECOMMENDATIONS AND GUIDELINES FOR EVALUATION OF PLATELET FUNCTION**

**In Vitro Evaluation of Platelet Biochemistry and Function**

The Food and Drug Administration (FDA) objective is to provide a guideline that will demonstrate that platelets which have been processed through a new collection procedures or new storage conditions can respond to a variety of stimuli equally well as platelets processed and stored by the FDA-approved blood banking practices.

*Morphology:* Platelet morphology should be visually inspected at different levels of resolution, starting with discs vs spheres estimate. The presence of different morphological forms should be quantitated, and finally the platelets should also be examined by electron microscopy.

*Biochemical status:* For stored platelets, in vitro tests of platelets have generally not correlated well with platelet performance in vivo. However, platelet cellular levels of ATP, glucose, and lactate should offer some indication of platelet performance. A drop in platelet count with an increase in the level of lactate dehydrogenase in medium can be used as a measure of cellular lysis. The pH of platelet suspension above 7.6 and below 6.2 at the end of the storage period has been shown to correlate with decreased in vivo performance.3,8

*Markers to determine percent of platelets activated by procedure:* Activation of platelets is associated with surface expression of the following surface antigens: GMP-140 (P-selectin, CD 62), CD 63, and the active form (fibrinogen-binding) of GP IIb/IIIa (detected by PAC-1), thromboglobulin and/or PF-4, and or soluble P-selectin released by activated platelets into the medium are platelet-specific proteins and can be measured as indicators of platelet activation.

*Physiologic responses:* The functional ability of platelets can be estimated by their response to osmotic stress and by the extent of agonist-induced shape change. Aggregation to increasing concentrations of physiologic agonists such as ADP, collagen, epinephrine, or to dual agonist combinations of ADP/epinephrine and ADP/collagen will give an idea of the responsiveness of the platelet. The presence the platelet serotonin uptake and agonist-induced serotonin secretion and agonist-induced expression of platelet activation markers such as GMP-140, will also evaluate the platelet physiologic responses. The platelet discoid shape as measured photometrically by the extent of shape change (ESC) and hypotonic shock response (HSR) have been reported to correlate well with in vivo viability. The ESC measures the initial photometric change that occurs within the first few seconds of adding a platelet agonist to plasma-rich protein (PRP). The HSR measures the ability of the osmotic pumps within the platelet to remain capable of pumping water out of the platelet after PRP has been treated with water.

These tests should be run as a paired comparison with platelets stored in the FDA-approved storage containers (i.e. stored in gas-permeable plastic bags, containing equal volumes, equal numbers of platelets and of white cells as the test platelets, on a rotator at 20–24). If an alternative storage medium other than plasma is used, the in vitro test conditions should mimic the conditions encountered by the platelets infused in vivo (i.e. resuspension in normal plasma). If non-plasma stored platelets are resuspended in plasma for in vitro testing and compared to plasma-stored platelets, the resuspending plasma should be equivalent to the plasma used for storage. That is, platelets resuspended in fresh-frozen plasma (FFP) should not be compared to platelets resuspended in plasma stored at room temperature for 5 days.
PLATELET SURVIVAL IN CIRCULATION

Prolonged in vivo circulation survival of transfused platelets has been considered as a sign of a fully functional, undamaged platelet. Any new procedure for platelet collection and storage which does not demonstrate significant changes in platelet responses on in vitro tests should be further tested for its effects on platelet in vivo survival. Recommendations on carrying out such tests have been published. Design of such tests should include normal volunteers receiving radiolabeled autologous platelets subjected to the novel treatment. Recent advances in double labeling of platelets with 111indium (111In) and 51chromium (51Cr) for a simultaneous comparison in a single recipient of novel vs conventional methods of platelet treatment have provided satisfactory results with less scatter in data points. The extent of data scatter will determine the number of volunteers that need to be tested to derive a clear conclusion about the effects of the test treatment.

A multi-tiered approach is usually used to evaluate platelet quality starting with a panel of in vitro measurements. Some investigators have been able to demonstrate a relationship between these assays and post-transfusion platelet viability measurements. However, even using those in vitro tests that have shown some ability to predict post-transfusion platelet viability, the correlation is often not very good, and some of the in vitro lesions are reversible following transfusion. Therefore, it is extremely important to ultimately document platelet quality with in vivo measurements of platelet recovery, platelet survival, and hemostatic efficacy; these measurements will be the focus of this review.

In vivo assessments are frequently performed using a two-step sequential process:
1. radiolabeled autologous platelet recovery and survival measurements in normal volunteers; and
2. transfusion experiments in thrombocytopenic patients. In thrombocytopenic patients, it is possible to assess both platelet viability by determining platelet increments and days to next transfusion, as well as platelet hemostasis by documenting the relationship between bleeding time and platelet count, determining hemorrhagic morbidity and mortality rates, and recording red cell transfusion requirements.

For reasons that have never been explained, there is a substantial amount of variability in the post-transfusion viability of platelets from different donors. Thus, the best experimental design to detect differences in platelet quality is to directly compare platelet product “A” to platelet product “B” using autologous platelets collected from the same donor. In normal volunteers, this is done by either simultaneously or sequentially preparing the two different autologous platelet products. For the simultaneous transfusion studies, one platelet product is labeled with 51Cr and the other with 111In prior to transfusion so that concurrent measurements can be done. For the sequential studies, the same isotope can be used. In patients, it is impractical to use the same donors’ platelets for simultaneous or even for sequential measurements. However, it is important to perform the sequential transfusions within a relatively short time period as changes in the patient’s clinical condition or medications may influence platelet transfusion outcomes.

CLINICAL HEMOSTATIC EFFICACY

Platelet efficacy in vivo has proven to be very difficult to define. Currently, there are no adequate FDA-approved clinical tests which will demonstrate platelet efficacy. In the past, bleeding time was thought of as the test of platelet efficacy, but numerous published studies have demonstrated the lack of correlation time between surgical bleeding and skin bleeding time and the variability of the bleeding time even with a single patient. New test methods will be further discussed in this chapter.

Current surrogate endpoints for platelet efficacy seems to be the availability of sufficient number of platelets in circulation (often taken as >20,000/μl) that have demonstrated a normal response to a battery of in vitro tests and normal in vivo half-life. The assumption is that a sufficient number of circulating and intact platelets will offer adequate protection against spurious intra-organ bleeding. Thus, the clinical performance (efficacy) of platelets obtained with novel methodologies should be evaluated by inclusion of these platelet products in clinical practice.

STUDIES ON PLATELET PRESERVATION

Random donor platelets, collected in PL732 CPD platelet bags, were obtained in a routine manner and...
were stored in the presently accepted preservative solution, citrate-dextrose-phosphate-plasma (CDP-P). These donor platelets underwent functional analyses with and without the experimental preservative solution [D-ribose, D-glucose, Hanks solution, Hepes solution, bovine serum albumin, tic anticoagulant peptide (TAP), sterile water; Table 7.1]. The following functional parameters were assessed at days 1 (day of collection) and day 5 of storage: platelet activation measuring P-selectin expression, adenine nucleotide content, as measured by adenosine triphosphate (ATP) levels and secretion of granule contents as measured by ATP release using bioluminescence, and response to agonists as monitored by platelet aggregation.

Table 7.1: Proprietary, ViaCyte, experimental preservative solution. Composition with concentration of components

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride dihydrate</td>
<td>0.18 gm/ml</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.3 gm/ml</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>0.09 gm/ml</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.4 gm/ml</td>
</tr>
<tr>
<td>Potassium phosphate monobasic</td>
<td>1.0 gm/ml</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>8.0 gm/ml</td>
</tr>
<tr>
<td>Sodium phosphate dibasic anhydrous</td>
<td>0.05 gm/ml</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>1.0 gm/L</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>150 nM/L</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Tic anticoagulant peptide</td>
<td>1 μM/L</td>
</tr>
</tbody>
</table>

Twenty-five bags of random donor PC were obtained from blood bank agencies. Approval by the internal review board was obtained for the use of these platelets for research purposes. Each bag of random donor platelets was subsequently divided into 10 equal aliquots. The aliquots were stored on a platelet agitator at room temperature in sterile polypropylene tubes with PL732 permeable covers. Two bags were excluded from this study due to technical problems. Within 2 hours from obtaining the PC, 20 μl of a 1 : 100 volume of the experimental preservative solution was added to nine separate aliquot tubes of PC. A 10th tube containing PC and no experimental preservative solution served as the control. All 10 samples were assayed for in vitro functional parameters.

Platelet activation was assessed with flow cytometry by monitoring P-selectin expression. Twenty μl of PC was diluted (100-fold) into a buffer containing 1 μM hirudin. The dilute blood samples were centrifuged for 10 minutes at 1000 g. Supernatants were discarded and the cell pellets were resuspended in buffer. Diluted whole-blood samples (100 μl) were activated with human thrombin (10.0 nM for 10 min, Haematologic Technologies, Essex Junction, VT), stained simultaneously for glycoprotein β3 and P-selectin (30 min), using mouse monoclonal immunoglobulin (IgG) against P-selectin (CD62) conjugated with phycoerythrin (PE) and mouse monoclonal IgG against glycoprotein IIb/IIIa (CD61) conjugated with flourescein (FITC, Becton-Dickinson, San Jose, CA). Each sample was fixed with the addition of 100 μl of 1 : 20 formalin for 30 minutes and then neutralized with 0.2 M Tris to a pH of 8.0. Samples were then diluted down to 2 ml for analysis. Samples were analyzed with a Partec (Muenster, Germany) CA3 flow cytometer.

Samples containing PC with and without the experimental preservative solution were assayed for rate of dense body ATP secretion and total platelet ATP content using a custom-designed luminometer. Platelet ATP secretion was measured by adding luciferase (1 mg/ml) and luciferin (10 μg/ml) to each sample. The luminescence generated by released ATP was compared with that of an ATP standard. For each assay, 40 μl of diluted (1 : 1000) platelets were mixed with 10 μl of luciferase reagent (0.5 mg/ml luciferase, 1.4 mg/ml luciferin, Sigma Chemical Co., St. Louis, MO) and placed in a photomultiplier tube compartment. Diluted platelets were activated with 50 μl of 10.0 nM human alpha-thrombin. Data were acquired using an OLIS (OLIS, Bogart GA) interface and software. Response was measured as rate of ATP secretion by plotting the slope of the secretion curve vs time. Total ATP content was measured as an amount of released ATP after lysis of cells by a detergent (50 μl 1 : 100 Triton X-100).

Without the ability to aggregate, platelets are unable to form effective hemostatic plugs, necessary to control active and chronic bleeding. Relative response of platelets to various agonists was measured photometrically. Each platelet sample was challenged with an agonist, such as adenosine diphosphate (ADP) or thrombin, to promote aggregation, as evidenced by clumping. Once a platelet aggregate is formed, more light is allowed to pass through the sample, which is measured by photometric analysis. An increased state of platelet aggregation is reflected as an increase in light emission with its relative intensity.
recorded by a turbidometer. Weak aggregation allows relatively less light to pass, and no response allows little or no light to pass above the baseline level.

Data analyses of the results of all samples assessing functional parameters are reported as percent change, mean, or observed change. When appropriate, the analysis of data for each assessed parameter between both groups (experimental preservative solution-treated vs untreated) was performed using a paired student t-test.

Results

Twenty-three bags of random PC were assessed for platelet activation, adenine nucleotide content, adenine nucleotide release and aggregation at days 0, 3 and 5. The mean pH of the control bags (CDP-P) at days 0 and 5 were 7.6 and 6.8, respectively. The mean pH of the bags with the addition of the experimental preservative solution was 7.6 and 7.0 at days 0 and 5, respectively.

Platelet Activation

Activation with surface expression of P-selectin on stored platelets is a predictor of in vitro viability. Once activated, platelets become refractory to further stimulation and lose their capacity to contribute effectively to hemostasis. At five days of storage at room temperature, 12.2 percent of platelets stored in the experimental preservative solution exhibited P-selectin expression at rest, and 64.2 percent upon activation with thrombin challenge, a difference of 52 percent. Platelets stored with CDP-P alone exhibited 44.4 percent P-selectin expression at rest and at day 5, suggesting significant activation during storage, and thrombin stimulation resulted in 47.9 percent P-selectin expression, a difference of only 2.5 percent (p ≤ 0.002, untreated vs treated). Table 7.2 reports the effect (percentage) of storage on activation of platelets with and without the experimental preservative solution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control1 % P-selectin</th>
<th>SD</th>
<th>Treated1 % P-Selectin</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 Basal</td>
<td>3.93</td>
<td>1.49</td>
<td>3.93</td>
<td>1.49</td>
</tr>
<tr>
<td>Day 0 Thrombin</td>
<td>81.6*</td>
<td>3.83</td>
<td>81.6*</td>
<td>3.83</td>
</tr>
<tr>
<td>Day 5 Basal</td>
<td>44.4</td>
<td>9.39</td>
<td>12.2</td>
<td>11.26</td>
</tr>
<tr>
<td>Day 5 Thrombin</td>
<td>47.9</td>
<td>6.49</td>
<td>64.2*</td>
<td>7.29</td>
</tr>
</tbody>
</table>

1 Difference between platelets activated by agonist challenge and those activated during storage (basal activation).

* Untreated vs ViaCyte, p≤0.002.

Adenine Nucleotide (ATP Secretion)

Table 7.3 reflects the effect of the experimental preservative solution on preserving intracellular ATP and the rate at which platelets secrete ATP upon agonist challenge. The results in Table 7.3 are represented as percent change from baseline (day 0). The experimental preservative solution maintained ATP levels throughout the storage period, while these levels became depressed in platelets stored in CDP solution alone (p ≤ 0.02, untreated vs treated). The maintenance of ATP levels provides the required cellular energy for cellular integrity, facilitate contraction and release of ADP, and to promote platelet aggregation.

Table 7.3. Platelet ATP secretion response and total ATP levels, percent (%) change from baseline (untreated vs ViaCyte treated)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days Storage</th>
<th>Rate1 (Δ Volts/min)</th>
<th>Total ATP2 (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>ViaCyte</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Untreated</td>
<td>5</td>
<td>2%</td>
<td>29%</td>
</tr>
<tr>
<td>ViaCyte</td>
<td>5</td>
<td>107%*</td>
<td>82%*</td>
</tr>
</tbody>
</table>

1 Rate of release of ATP from stored platelets in response to 10 nM alpha thrombin

2 Total ATP released from stored platelets by lysing the cells with Triton X-100

Platelet Aggregation

Platelet aggregation studies were performed in response to ADP and thrombin challenges. Typical ADP-induced aggregation response for control platelets was lost by day 5; whereas, ADP-induced aggregation response for the experimental preservative solution-treated platelets was intact after 5 days storage (data not shown). Likewise, thrombin challenge produced similar results as found with the ADP challenge. The amplitude of day 5 response of
the treated platelets was typically less than the amplitude achieved on day 1; however, the non-treated platelets failed to induce any level of aggregation response at 5 days of storage.

**DISCUSSION OF PLATELET PRESERVATION METHODS**

Current transfusion medicine practice permits platelets to be stored no longer than 5 days during which time platelets may lose a significant degree of hemostatic activity. It is estimated that by and large, 15 percent of platelet units collected are discarded. Over the past four decades, attempts have been made to develop methods for preserving platelets for longer periods of time with improved functional capabilities. This work, the majority of which was performed in the 1960s and 1970s, culminated in the currently accepted 5 days of storage in CDP solution at room temperature. Murphy and Gardner demonstrated that room temperature storage helped protect hemostatic function better than cold storage of the platelet concentrates. Additional studies revealed that platelet function could be improved further with storage bags having improved gas permeability, preservative solutions that contained nutrients/metabolites, through better control of pH and the addition of protease inhibitors. For example, reports in the literature have recommended various novel preservative solutions, such as glucose-free (platelet additive solution, PAS) Setosol, PlasmaLyte, or citrate/acetate base cocktails. Up until the late 1960s, platelets were transfused only in whole blood. A milestone in platelet transfusion was reached by introducing plastic blood bags with the capability of interconnections to several satellite bags. This system significantly minimized the obvious risk of bacterial contamination. With the introduction of newer plastic, gas permeable (O₂, CO₂) PL732 bags in the middle 1980s, platelets were allowed to be stored for 7 days in the United States, but a higher occurrence of bacterial contamination urged the Food and Drug Administration (FDA) to reduce the storage period to 5 days.

During storage, platelets develop storage-mediated lesions and exhibit a progressive loss of viability, deterioration in pH, or compromised intracellular metabolic components, even though they may have the ability to increase corrected count increments in vivo. This loss in viability is reflected in their loss of membrane integrity, inability to maintain shape, and exhibit altered HSR. Further, a significant progressive decrease in hemostatic properties occurs, as evidenced by decreased platelet aggregation response, decreased adenine nucleotide concentration, increase in storage-induced activation, loss of expression or affinity of surface receptors, activation of complement, and/or change in intracellular calcium concentration leading to a cascade of events promoting platelet activation during storage. The majority of platelets undergo some degree of activation during procurement, processing or storage leading to in vivo functional compromise.

Platelets utilize energy for a variety of intracellular biochemical reactions associated with their role in hemostasis. Unlike many other cells, platelets do not contain creatine or creatine kinase and rely on large amounts of intracellular, stored glycogen to fuel glycolysis. The principal source of energy for platelets is the hydrolysis of ATP. ATP is essential for signal transduction and, once released, creates calcium cross-linkages. In order to maintain adequate platelet function, platelets must generate ATP continuously to meet their energy needs. Many of the current platelet additive solutions have focused on the chemical pathways of glycolysis and oxidative phosphorylation. In glycolysis, one molecule of glucose is converted to a small net gain in ATP molecules. In oxidation, glucose, fatty acids or amino acids enter the citric acid cycle and is converted to CO₂ and H₂O. With a sufficient supply of oxygen, oxidative phosphorylation is more efficient than glycolysis, producing up to 36 molecules of ATP per molecule of glucose. Lastly, platelets also have the capability to generate ATP by means of the pentose phosphate pathway.

Our experimental preservative solution was designed to maintain functional integrity of blood components by increasing intracellular metabolic energy supplies, thereby protecting platelets from storage-induced lesions. Although this study contained bovine serum albumin and tic anticoagulant peptide (TAP), subsequent studies have demonstrated that removal of albumin and TAP altogether has not altered the assessed findings. It would be beneficial to not include bovine albumin as it may introduce some unknown pathogens.
ViaCyte-treated platelets maintained high levels of ATP, responded to agonists with P-selectin expression, and maintained aggregation response, unlike the currently utilized, approved preservation solution, CPD-P. ViaCyte may provide the opportunity to safely and economically reduce the dose of transfused platelets while optimizing clinical outcome. Further, the noted improved functional viability of platelets stored at 5 days with ViaCyte stimulates future research aimed at extending the shelf-life, which would significantly reduce outdates, provide more functionally capable platelets, and help eliminate the shortage of platelets worldwide.

**Device for Monitoring Platelet Function**

Medical device manufactures are currently in pursuit of a point-of-care (POC) device that would serve to provide rapid detection of the functional capability of platelets for therapeutic applications. It is our belief, that these medical devices should be available for platelet function monitoring, just as every patient on heparin therapy is monitored with ACT measurements. Every patient on antiplatelet therapy would benefit from measurement with the platelet function measurement. Rapid POC platelet function would be a benefit for the following: Cath Lab (angioplasty) procedures, GP IIb-IIIa antiplatelet sensitivity, aspirin sensitivity, neurological, dental, bleeding time assessment, transfusion medicine, cardiopulmonary bypass, patient risk markers, heart valve patients.

The SUBC CardioVascular Inc. in Rochester Minnesota has developed a Platelet Time Detection System that is intended for rapid assessment of platelet function and platelet function inhibition. The newly developed platelet function test is capable of assessing the effect of platelet inhibition pharmacology for antiplatelet agents such as Aspirin, Plavix, and the GP IIb-IIIa inhibitors such as Integrilin and ReoPro. The device consists of a hardware unit that is built to FDA guidelines with a complete device history file. The device is capable of measuring platelet function in a rapid fashion/real-time POC setting using either an intra-arterial or an intravenous catheter. In addition to on-line real-time monitoring, the device has been specifically designed to provide discrete sample measurements for laboratory benchtop use. Accompanying the hardware is the requirement of the disposable portion of the device.

Clot time measurements have been available for anticoagulation monitoring since the late 1960s with the introduction of ACT measurements for heparin management, as well as the previously established routine laboratory clot time tests such as PTT, TT and APTT. The routine assessment of these parameters has allowed accurate, rapid and safe coagulation management. In recent years, the use of antiplatelet agents has intensified. Reasons for this are the increased use of implantable devices such as heart valves and stents, increase safety and efficacy of antiplatelet agents, increase in the number of patients treated for unstable angina, and increased understanding of the underlying mechanisms associated with platelet pathophysiology and biochemistry. However, with the increased use of antiplatelet agents, there has not been an introduction into the market of a POC platelet time function test.

The degree of patient-to-patient variability for individuals on various antiplatelet agents is analogous to the variation observed by the prolongation of ACT for patients on heparin therapy. However, until now, there has not been a POC platelet time test that can provide the clinician the same type of knowledge base as that received from ACT measurements. The SUBC Cardiovascular Inc. intends to introduce the first POC platelet test that will provide the clinician crucial platelet function information.

**Detection of Platelet Function and Platelet Function Inhibition**

Blood platelets provide a cellular and enzymatic method for cessation of bleeding in normal hemostasis. The blood platelet is responsible for providing the “sticking” of cells at the site of injury. The ensuing platelet adhesion and platelet plug formation are responsible for the stoppage of bleeding and the initiation of wound healing. However, certain individuals are predisposed to enhanced platelet plug formation and are, therefore, at risk for events such as blood clot formation or stroke. Patients at risk are often placed on antiplatelet drugs. In addition, patients who have implantable devices such as stents and heart valves are also at risk of clot formation and embolization formation initiating at the site of the device, which also place the patient at risk. Therefore, patients with implantable devices are placed on antiplatelet and anticoagulant drugs to prevent possible coagul-
ation or stroke. The problem at hand is that the anti-
platelet drugs have a large patient-to-patient
variability; and in many instances, patients are
refractory to some antiplatelet drugs. There is no
current test on the market that can ensure that platelet
function has been inhibited for patients at risk. The
SUBC Platelet Detection System provides a device that
can determine if platelet function has been inhibited,
thereby allowing the attending physician to adjust
pharmacologic parameters prior to intervention. Such
customized therapy will lead to reduced risk of clot
formation and embolization.

Platelet plug formation is assessed by bringing the
blood into contact with a foreign surface in a manner
that induces platelet adhesion and ensuing accretion
which in turn leads to the formation of a platelet plug.
In this platelet plug device, the blood is drawn into
an open-ended tube such as a catheter. Within the
open tube, a foreign material that attracts platelets to
adhere is positioned within the center of the tube and
held into position with a fixed holder at the other end.
A pump is used to draw a defined volume of blood,
at a defined flow rate and defined shear rate, into the
open tube, and over the foreign material. The pump
can be used either in a single or in a bi-directional
mode; however, it is required that blood continuously
be brought into contact with the foreign material. As
platelets are brought into contact with the foreign
material, the platelets change shape, adhere, release,
and accrete. The platelet accretion and aggregation
continue until the lumen of the tube is occluded with
the platelet plug. The use of a pressure transducer is
one means of determining when complete occlusion
of the lumen is obtained.

The device can be used on the arterial or venous
side of a patient to provide “real-time” platelet plug
formation information. Alternatively, the device could
be used as a bedside stand alone instrument in which
sample of blood drawn from a patient line could be
introduced into the test configuration. This platelet/
coagulation activation, monitoring device, also could
be used to monitor the functional capabilities of
platelets prior to transfusion of PCs for therapeutic
purposes or for assessing in vivo function of platelets
after transfusion. Results of our preliminary studies
on this devise, using animal models are presented in
Tables 7.4 to 7.6.

<table>
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<th>Day</th>
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<td>.35</td>
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<td>Post 7 days</td>
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<table>
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<td>Pre</td>
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</tr>
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<td>3</td>
<td>5.67</td>
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<td>7</td>
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<tr>
<td>Day 3</td>
<td>18.5</td>
</tr>
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Administration of Aspirin or Ticlopidine alone or
in combination, significantly increased the clotting
time in these studies. Inhibition of platelet function or
the degree of platelet function can be assessed in either
whole-blood samples or by intravascular approaches
intravenously by utilizing the above described Platelet
Detection System in which blood is continually
brought into contact with a foreign material that
preferentially recruits the attachment and eventual
platelet plug formation. Studies are in progress to
evaluate this test system to monitor functional
capabilities of stored platelets. We are also developing
similar test systems capable of monitoring platelet
activation, using expression of activation markers such
as P-selectin or released ATP.

**CONCLUSION**

Blood and blood products have been successfully used
for transfusion therapy for several decades. In the last
few years, there has been an increased demand for
random donor platelet concentrates as well as single
donor apheresis platelets. In spite of the large-scale use of stored platelets, little improvements have been made in the preservation of function of platelets during the storage period. Similarly, there are hardly any new methodologies to detect the functional capabilities of platelets prior to therapeutic applications or for monitoring the efficacy of the drug therapy. Furthermore, there is a great need for point of care testing devices which provide rapid, accurate information on the function of platelets as well as coagulation profile. In this article, we have reviewed briefly some of the historical developments in the platelet preservation methodologies, described a new platelet preservative, provided some relevant data obtained using this new preservative. We also have reviewed the void that exists in the POC testing methodologies that exist currently and the immediate need for such a test system under a variety of clinical situations. We have briefly described a test system that we are developing to meet the requirements under a variety of clinical conditions including for testing PCs before transfusion to patients in need. We hope that this overview stimulates the researchers in this area, to renew their efforts to develop new “novel” preservatives for improving the quality of cells stored for transfusion therapies. We also hope renewed efforts will lead to the development of rapid detection systems for assessing the functional status of circulating platelets and the coagulation cascade.

REFERENCES

The HLA System and Transfusion Medicine

S Yoon Choo

THE HLA SYSTEM

The genetic loci involved in the rejection of foreign organs are known as the major histocompatibility complex (MHC), and highly polymorphic cell surface molecules are encoded by the MHC. The human MHC is called the human leukocyte antigen (HLA) system because these antigens were first identified and characterized using alloantibodies against leukocytes.1

The HLA system has been well known as transplantation antigens, but the primary biological role of the HLA molecules is in the regulation of immune response.2

Genomic Organization of the Human MHC

The human MHC maps to the short arm of chromosome 6 (6p21) and spans approximately 3,600 kilobases of DNA.3 The human MHC can be divided into three regions. The class I region contains the classical genes (HLA-A, HLA-B, HLA-C), the nonclassical genes (HLA-E, HLA-F, HLA-G), pseudogenes (HLA-H, HLA-J, HLA-K, HLA-L), and gene fragments (HLA-N, HLA-S, HLA-X).4 The HLA-A, HLA-B, and HLA-C loci encode the heavy alpha chains of class I antigens. Some of the nonclassical class I genes are expressed with limited polymorphism, and their functions are not well known at this time.

The class II region consists of a series of subregions, each containing A and B genes encoding α and β chains, respectively.4 The DR, DQ, and DP subregions encode the major expressed products of the class II region. The DR gene family consists of a single DRA gene and nine DRB genes (DRB1 – DRB9). Different HLA haplotypes contain particular numbers of DRB loci. The DRB1, DRB3, DRB4, and DRB5 loci are usually expressed, and the other DRB loci are pseudogenes. The DRA locus encodes an invariant α chain and it binds various β chains. HLA-DR antigen specificities (i.e. DR1 to DR18) are determined by the polymorphic DRβ1 chains encoded by DRB1 alleles. The DQ and DP families each have one expressed gene for α and β chains and additional pseudogenes. The DQA1 and DQB1 gene products associate to form the DQ molecule, and the DPA1 and DPB1 products form DP molecules.

The class III region does not encode the HLA molecules but contains genes for complement components (C2, C4, factor B), 21-hydroxylase, and tumor necrosis factors (TNFs).3

HLA Haplotypes

The HLA genes are closely linked and the entire MHC is inherited as an HLA haplotype in a mendelian fashion from each parent. Recombination within the HLA system occurs with a frequency less than 1 percent, and it appears to occur most frequently between the DQ and DP loci. The segregation of HLA haplotypes within a family can be assigned by family studies. Two siblings have a 25 percent chance of being genotypically HLA identical, a 50 percent chance of being HLA haploidentical (sharing one haplotype), and a 25 percent chance that they share no HLA haplotypes.

Possible combinations of antigens from different HLA loci on an HLA haplotype are enormous, but some HLA haplotypes are found more frequently in certain populations than expected by chance. This phenomenon is called the linkage disequilibrium. For
example, HLA-A1, B8, DR17 is the most common HLA haplotype among Caucasians, with a frequency of 5 percent.

**Tissue Expression of HLA**

The HLA class I molecules are expressed on the surface of almost all nucleated cells. They can also be found on red blood cells and platelets. Class I molecules on the mature red cell surface likely derive from endogenous synthesis by erythroid precursor cells and also from adsorption of soluble antigens present in plasma. The Bg serological specificities in immunohematology represent various HLA antigens. The HLA class I molecules present on the platelet surface probably derive from megakaryocytes and also from adsorption of soluble antigens from plasma.

Class II molecules are expressed exclusively on B lymphocytes, antigen-presenting cells (monocytes, macrophages, and dendritic cells), and activated T lymphocytes.

**Structure and Polymorphism of HLA Molecules**

Class I molecules consist of glycosylated heavy chains of 44,000 to 45,000 daltons (44–45 kDa) encoded by the HLA class I genes and a noncovalently bound extracellular 12 kDa β2-microglobulin (β2m). Human β2m is invariant and is encoded by a non-MHC gene. The class I heavy chain has three extracellular domains (α1, α2, and α3), a transmembrane region, and an intracytoplasmic domain. The α1 and α2 domains contain variable amino acid sequences, and these domains determine the serologic specificities of the HLA class I antigens. These two heavy chain domains form a unique structure consisting of a platform of eight antiparallel β strands and two antiparallel α-helices on top of the platform. A groove is formed by the two α-helices and the β-pleated floor, and this is the binding site for processed peptide antigen. The class I peptide-binding groove accommodates a processed peptide of 8 to 10 (predominantly nonamers) amino acid residues.

The products of the class II genes DR, DQ, and DP are heterodimers of two noncovalently associated glycosylated polypeptide chains: α (30–34 kDa) and β (26–29 kDa). An extracellular portion composed of two domains (α1 and α2, or β1 and β2) is anchored on the membrane by a short transmembrane region and a cytoplasmic domain. The extent of class II molecule variation depends on the subregion and the polypeptide chain. Most polymorphisms occur in the first amino terminal domain of DRB1, DQB1, and DPB1 gene products. The three-dimensional structure of the HLA-DR molecule is similar to that of the class I molecule. The α1 and β1 domains form an antigen-binding groove. The class II groove is more open so that longer peptides (12 amino acids or longer) can be accommodated.

**Peptide Presenting Role of HLA Molecules**

T cells recognize processed peptides on the cell surface. Zinkernagel and Dougherty demonstrated in 1974 that T lymphocytes must have the same MHC molecules as the antigen-presenting cell to induce immune response. The phenomenon that peptides are bound to MHC molecules and these complexes are recognized on the cell surface by the T-cell receptor is called the MHC restriction. During the T-cell maturation in the thymus, T lymphocytes are educated and selected to recognize the self-MHC molecules, and thereafter MHC molecules play a role as determinants of immune response.

The peptide-binding specificities of HLA molecules are determined by a relatively limited number of amino acid residues located in the peptide-binding pockets. Fine structure of these pockets changes depending on the nature of the amino acids within the groove. Different HLA molecules show characteristic amino acid residue patterns in the bound peptiDES. Amino acid residues that are located at particular positions of the peptides are thought to act as peptide’s anchoring residues in the peptide-binding groove.

The nature and source of peptides that will bind to class I or class II molecules are different. Class I-restricted T cells recognize endogenous antigens synthesized within the target cell, whereas class II-restricted T cells recognize exogenously derived antigens.

There are two forms of T-cell receptor (TCR): polypeptide heterodimers composed of disulfide-linked subunits of either αβ or γδ. The αβ TCR is present on more than 95 percent of peripheral blood T cells. The TCR molecule is associated on the cell surface with a complex of polypeptides, CD3. During
the recognition process between TCR and HLA-peptide complex, accessory molecules on T lymphocytes are enhancing the interaction between T lymphocytes and HLA molecules. The CD4 molecule interacts with a class II molecule on the APC, and the CD8 molecule interacts with a class I heavy chain on the target cells. Structural studies show that the overall mechanism of TCR recognition of self-MHC and allogeneic MHC molecules is similar.15

Natural killer (NK) cells are a subset of lymphocytes (10–30 percent of peripheral blood lymphocytes) that lack both CD3 and TCR and exert cytotoxicity.16 NK cell recognition is not MHC restricted. NK cells have been known to recognize the loss of expression of HLA class I molecules (missing self) and destroy cells with decreased expression of class I molecules such as some tumors and virally infected cells. Other cells with normal MHC class I expression can still be NK targets if they provide appropriate signals to activating NK cell receptors. Many different NK cell receptors have been identified and majority of their ligands are HLA class I molecules. NK cells are regulated by both inhibitory and activating signals resulting from the NK cell receptor-ligand binding.16

**CLINICAL HLA TESTING**

The HLA testing in the transplant workup includes the HLA typing of the recipient and the potential donor, screening and identification of preformed HLA antibodies in the recipient, and detection of antibodies of the recipient that are reactive with lymphocytes of a prospective donor (crossmatch).

**SeroLogic Typing of HLA Antigens**

The complement-mediated microlymphocytotoxicity technique has been used as the standard for serologic typing of HLA class I and class II antigens.17,18 The HLA typing sera are mainly obtained from multiparous alloimmunized women, and their HLA specificities are determined against a panel of cells with known HLA antigens. Some monoclonal antibody reagents are also used.

Peripheral blood lymphocytes (PBLs) express HLA class I antigens and are used for the serologic typing of HLA-A, HLA-B, and HLA-C. The HLA class II typing is done with isolated B lymphocytes because these cells express class II molecules. PBLs are commonly isolated by density-gradient separation; various techniques are available to isolate B lymphocytes, including B-specific monoclonal antibody-coated magnetic beads.

Formal assignments of serologically defined antigens are given by the World Health Organization HLA Nomenclature Committee, which is responsible for the nomenclature of the HLA system.4

**Molecular Typing of HLA Alleles**

Studies of the HLA system using monoclonal antibodies, electrophoretic gel analysis, cellular assay, and molecular techniques have revealed that the extent of HLA polymorphism is far higher than previously known antigen specificities (Table 8.1). Serologically undistinguishable variants or subtypes of HLA class I antigens were identified by these methods, and it was demonstrated that these variants are different from the wild type by a few (usually one to five) amino acids, but these can be differentially recognized by alloreactive or MHC-restricted T lymphocytes.19 Clinical molecular typing has been developed to distinguish serologically undistinguishable but functionally discrete HLA alleles.

Molecular typing of HLA class II genes based on the polymerase chain reaction (PCR) using thermostable DNA polymerase, called Taq I polymerase, has become available to amplify and study HLA genes.20 PCR-based clinical HLA typing was first developed using sequence-specific oligonucleotide probe (SSOP) methods.21 The hypervariable exon 2 sequences encoding the first amino terminal domains of the DRB1, DQB1, and DPB1 genes are amplified from genomic DNA by PCR reaction. Based on the HLA sequence database, a panel of synthetic oligonucleotide sequences corresponding to variable regions of the gene are designed and used as SSOP in

<table>
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<th>Locus</th>
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<tr>
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<td>559</td>
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<td>HLA-C</td>
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<td>150</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>17</td>
<td>362</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>105</strong></td>
<td><strong>1,374</strong></td>
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hybridization with the amplified PCR products. Alternatively, polymorphic DNA sequences can be used as amplification primers; and in this case, only alleles containing sequences complementary to these primers will anneal to the primers and amplification will proceed. This strategy of DNA typing is called the sequence-specific primer (SSP) method, and it detects sequence polymorphism at given areas by the presence of a particular amplified DNA fragment. High-resolution HLA-DRB1 allele typing has been routinely used to support unrelated donor marrow transplantation.22

The development of HLA class I allele typing has been much behind that of class II. The class I polymorphism is located in the two domains, \( \alpha_1 \) and \( \beta_2 \)(requiring amplification of two exons and an intervening intron), and there are many more polymorphic sequences (requiring more probes or primers than in class II). These differences made it more challenging to develop molecular typing strategies for class I.

Actual DNA sequencing of amplified products of multiple HLA loci is increasingly used as clinical HLA typing in the unrelated donor hematopoietic stem cell transplantation. The HLA alleles are designated by the locus followed by an asterisk, a two-digit number corresponding to the antigen specificity, and the assigned allele number. For example, \( \text{HLA-A}^*0210 \) represents the tenth HLA-A2 allele encoding a unique amino acid sequence within the serologically defined HLA-A2 antigen family.

The HLA Antibody Screening and Lymphocyte Crossmatch

Preformed HLA antibodies can be detected by testing the patient’s serum against a panel of lymphocytes with known HLA specificities. The complement-mediated microlymphocytotoxicity technique has been the standard, and the antihuman globulin (AHG) method provides higher sensitivity.23 This test is called HLA antibody screening and the results are expressed as the percentage of the panel cells that are reactive; this is called the percent panel reactive antibody (percent PRA). For instance, if 10 of 40 different panel cells are reactive with a serum, the PRA is 25 percent. With a panel of well-selected cells representing various HLA antigens, antibody specificities can sometimes be assigned. This information is particularly important for the organ transplant candidate to predict the chance of finding a compatible or crossmatch-negative cadaver donor and to avoid specific mismatched HLA antigens in the donor. When a potential donor becomes available, a final crossmatch is performed between the recipient’s serum and the donor’s lymphocytes to determine the compatibility. The positive crossmatch results are predictive of the risk of graft rejection.24,25 Antibodies to both HLA class I and class II antigens are detrimental.

Alternative methods based on enzyme-linked immunosorbent assay (ELISA) and flow cytometry are also available for HLA antibody screening and antibody specificity identification. In recent years, a lymphocyte crossmatch using flow cytometry has been widely used. This technique presumably offers higher sensitivity and may be more predictive of allograft rejection.

THE HUMAN MINOR HISTOCOMPATIBILITY ANTIGENS

Minor histocompatibility antigens are naturally processed peptides derived from normal cellular proteins that associate with HLA molecules.26 Minor histocompatibility antigens are inherited and have allelic forms. The number of minor histocompatibility loci is probably high, and the extent of polymorphism for each locus is not known. Examples include the male-specific H-Y antigens and a series of HA antigens. Minor histocompatibility antigens may affect the outcome of hematopoietic stem cell and solid organ transplants. Minor histocompatibility antigen disparity can be associated with GVHD in HLA-identical transplants (e.g. H-Y antigen in a male recipient and a female donor who has been immunized by pregnancy).27

THE HLA SYSTEM AND TRANSPLANTATION

The HLA-A, HLA-B, and HLA-DR have long been known as major transplantation antigens. The role of HLA-C and other class II molecules is being actively investigated, especially in hematopoietic stem cell transplantation.

Both T-cell and B-cell (antibody) immune responses are important in graft rejection.28 T lymphocytes
recognize donor-derived peptides in association with the HLA molecules on the graft. The graft may present different allelic forms of the minor histocompatibility antigens, and the donor’s HLA molecules may present a different set of peptides to the recipient’s T-cells. T-cell alloreognition plays an important role in acute and chronic rejection.

Antibodies to the graft fix complement and cause damage to the vascular endothelium, resulting in thrombosis, platelet aggregation, and hemorrhage. Hyperacute rejection occurs in patients who already have antibodies specific to a graft. Antibodies against ABO blood group and preformed HLA antibodies induce hyperacute rejection. The HLA alloimmunization can be induced by blood transfusions, pregnancies, or failed transplants. Hyperacute rejection can be avoided in most cases by ABO-identical or ABO-major compatible transplantation and by confirming negative lymphocyte crossmatching.

### Solid Organ Transplantation

Solid organs can be donated by cadaveric donors, living related donors, or living unrelated donors. In solid organ transplantation, blood group ABO system is the most important major histocompatibility antigen. Natural anti-A and anti-B antibodies cause hyperacute rejection because ABO antigens are expressed on endothelial cells. Kidney transplantation is performed only from ABO-identical or ABO-major compatible donors. Preformed HLA antibodies also cause hyperacute rejection. The problem of hyperacute rejection can be predicted by a positive donor lymphocyte crossmatch and can be prevented when transplantation is performed from a donor whose lymphocytes are not reactive with recipient’s serum. The presence in the recipient of preformed HLA antibodies reactive with a donor’s lymphocytes is a contraindication to kidney transplantation.

For heart transplant candidates, initial HLA antibody screening is routine and prospective lymphocyte crossmatching is usually performed for HLA alloimmunized patients. Pretransplant crossmatching is not performed prior to liver transplant because of the urgent need of organs and the uncertain benefits of a crossmatch-negative transplant.

The benefits of HLA matching are well established in renal transplantation. There is a clear relationship between the degree of HLA matching and kidney graft survival in transplants from living related donors. Better results are obtained from an HLA-identical sibling donor than with HLA-haploidentical parents, siblings, or children. Kidney transplantation from a living unrelated donor, commonly a spouse, shows graft survival superior to cadaveric transplantation (except for six-antigen match) despite a greater degree of HLA mismatch. These favorable results are probably the result of shorter ischemic time and less renal damage.

The influence of HLA matching on the survival of liver and thoracic organs is yet uncertain, even though there is some evidence that the outcome of heart transplantation may be influenced by the degree of HLA matching.

### Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation is used to treat hematologic malignancy, severe aplastic anemia, severe congenital immunodeficiencies, and selected inherited metabolic diseases. The sources of hematopoietic stem cells are bone marrow, mobilized peripheral blood stem cells, and umbilical cord blood.

ABO blood group incompatibility is not a clinically significant barrier to stem cell transplantation. The HLA system is the major histocompatibility antigen in stem cell transplants, and the degree of HLA matching is predictive of the clinical outcome. The HLA mismatch between a recipient and a stem cell donor represents a risk factor not only for graft rejection but also for acute graft-versus-host disease (GVHD) because immunocompetent donor T cells are introduced to the recipient. T-cell depletion of donor marrow results in lower incidence of acute GVHD but higher incidence of graft failure, graft rejection, disease relapse (loss of the graft-versus-leukemia effect), impaired immune recovery, and later complication of Epstein-Barr virus-associated lymphoproliferative disorders.

The risk of graft rejection or failure is especially higher in patients with severe aplastic anemia because these patients are frequently alloimmunized by multiple blood transfusions prior to transplant and their preconditioning regimen is less intensive than that for leukemia.
The best compatible stem cells are from an identical twin and a genotypically HLA-identical sibling. An HLA-identical sibling is found in approximately 25 percent of patients. For those who do not have a matched sibling, an alternative related family member who is HLA haploidentical and partially mismatched for the nonshared HLA haplotypes may serve as a donor, but these transplants have a higher risk of developing acute GVHD and graft rejection or failure.36

When an HLA-matched or partially mismatched acceptable related donor is not available, phenotypically matched unrelated donors can be considered.37 The chance of finding an HLA-matched unrelated donor depends on the patient’s HLA phenotype.38

Unrelated donor transplants are associated with an increased incidence of acute GVHD and graft failure/rejection compared to HLA-matched sibling transplants. Such an increase may result partly from mismatch in HLA alleles and from minor histocompatibility antigens.27,39,40 For this reason, HLA-A, B, C, and DRB1 allele matching is strongly recommended for unrelated donor transplants. Some patients do not find a perfectly allele-matched unrelated donor for multiple loci and a partially mismatched unrelated donor can still be considered for transplant for some selected patients. Further studies are needed to better understand the unfavorable effects of mismatched alleles at different HLA loci on graft failure/rejection, GVHD, and survival.

**Transfusion Practice in Stem Cell Transplantation**

Transfusion policy should include measures to prevent alloimmunization in all potential stem cell transplant candidates. All transplant candidates and recipients should receive leukoreduced cellular components in order to prevent or reduce the risk of HLA alloimmunization. Transfusion from blood relatives should be avoided for a patient who is a candidate for a stem cell transplant. The minor histocompatibility antigens are inherited independently of the MHC region, and thus any transfusions from blood relatives could lead to an exposure to possibly relevant antigens.

**THE HLA SYSTEM IN TRANSFUSION THERAPY**

The HLA system can cause adverse immunologic effects in transfusion therapy. These effects are primarily mediated by donor “passenger” leukocytes contained in the cellular blood components. The HLA antibodies can be induced from previous alloimmunization episodes and can cause platelet immune refractoriness, febrile nonhemolytic transfusion reaction, and transfusion-related acute lung injury.

**HLA Alloimmunization**

Multiparous women are frequently alloimmunized to HLA and their HLA antibodies may persist or become gradually undetectable. Primary HLA alloimmunization by blood transfusion is caused by the leukocytes in the cellular blood products.41 The HLA antibodies found in alloimmunized patients are usually broadly reactive.

The incidence of HLA alloimmunization following transfusions can vary with the patient’s diagnosis and therapy.42 The HLA antibodies can be detected in 25 to 30 percent of transfused leukemic patients and can be present in as high as 80 percent of aplastic anemia patients. Leukemic patients are usually transfused while receiving intensive chemotherapy, which induces immunosuppression and this reduces the incidence of transfusion-induced alloimmunization. Severe aplastic anemia patients who had received blood transfusions have a higher incidence of graft rejection following stem cell transplantations.43

Leukocyte reduction to less than $5 \times 10^6$ can prevent or reduce the development of primary HLA alloimmunization.44 Leukoreduction can be achieved for platelet and red blood cell components by the use of third-generation leukocyte reduction filters. Leukoreduced platelet products can be collected from certain models of apheresis equipment. The incidence of HLA antibody development, however, is not decreased or delayed in patients with previous pregnancies by the leukocyte reduction filtration.45 It appears that the secondary HLA immune response cannot be prevented by the degree of leukocyte reduction currently available.

**Refractoriness to Platelet Transfusion**

Platelet refractoriness is a consistently inadequate response to platelet transfusions. There are immune and nonimmune causes for poor post-transfusion increments.46,47 Practically, platelet immune refractoriness can be suspected if the 1-hour post-transfusion
platelet recovery is less than 20 percent of expected increment and there are no known nonimmune adverse factors. The major nonimmune adverse factors are fever, splenomegaly/hypersplenism, antibiotics (amphotericin B, vancomycin, ciprofloxacin), disseminated intravascular coagulation, infection, sepsis, marrow transplantation, veno-occlusive disease, and bleeding at the time of transfusion.

The HLA class I antigens are expressed on platelets and the development of antibodies to HLA or platelet-specific antigens can cause immune destruction of transfused platelets, resulting in a refractoriness to random donor platelet transfusions. Definite diagnosis of platelet immune refractoriness is confirmed if antibodies against HLA and/or platelet-specific antigens are detected and non-immune causes of platelet refractoriness are ruled out. In reality, most patients with suspected refractoriness are found to have one or more concurrent non-immune adverse factors. When patients are suspected for immune refractoriness, HLA and platelet-specific antibody screening is performed. Presence of HLA and/or platelet-specific antibodies can be detected by using various techniques including lymphocytotoxicity test, flow cytometry, ELISA, monoclonal antibody-specific immobilization of platelet antigens assay (MAIPA), mixed passive hemagglutination assay (MPHA), and solid phase assay. Most refractory patients are immunized to HLA, and immunization to platelet-specific antigens is much less frequent.

Once the clinical and laboratory diagnosis of immune refractoriness is made, the use of special platelet products is indicated. Most patients who are refractory to random donor platelets because of HLA antibodies respond to HLA-matched platelets. HLA-matched siblings or HLA-haploidentical family members can donate platelets by apheresis, but to prevent alloimmunization to minor histocompatibility antigens, these blood-related donors should not support patients who are candidates for a stem cell transplant. If the specificity of the patient’s antibodies can be determined, donors who are negative for corresponding HLA antigens can be selected.

A number of techniques have been tried to determine platelet compatibility. Platelet cross-matching using a solid-phase red cell adherence technique has been developed. This technique will detect platelet antibodies against HLA class I and platelet-specific antigens. Collected platelet apheresis units are crossmatched with the patient’s serum, and crossmatch compatible units are identified. The efficacy of crossmatched platelets may be as good as HLA-matched platelets in some patients. The main advantage of using platelet-crossmatched products over HLA-matched platelets is that these units are immediately available for transfusion.

Since primary HLA alloimmunization caused by platelet transfusion is induced by contaminating leukocytes, this potential problem can be prevented or reduced by the use of the third-generation leukoreduction filter. In patients with previous pregnancies, leukocyte reduction does not reduce the incidence of HLA antibody development and platelet refractoriness. Most previously pregnant patients appear to develop HLA antibodies by a secondary immune response during transfusion therapy. Platelets per se, soluble HLA antigens, residual leukocytes, or leukocyte fragments escaping leukoreduction filtration may be able to induce a secondary HLA immune response. Prevention of alloimmunization is indicated for patients who are expected to need long-term platelet transfusions. Experience of the universal prestorage leukoreduction demonstrated decreased incidence of alloimmune platelet transfusion refractoriness.

Transfusion-Associated Graft-versus-Host Disease

When functionally competent allogeneic T lymphocytes are transfused into an individual who is severely immunocompromised, these T lymphocytes are not removed and can mount an immune attack against the recipient’s cells, resulting in the development of GVHD (TA-GVHD). TA-GVHD is not common and typically occurs in patients with congenital or acquired immunodeficiencies or immunosuppression that affects T lymphocytes.

TA-GVHD has also occurred in patients without apparent evidence of immunodeficiency or immunosuppression. The majority of these studied cases involved a blood donor who was homozygous for one or more HLA loci for which the recipient was heterozygous for the same antigen and a different one. This relationship can be called a one-way HLA mismatch in the GVHD direction and a one-way HLA
match in the rejection direction. As a result, the donor’s cells will not be recognized as foreign by the recipient’s lymphocytes, while the donor’s lymphocytes will recognize HLA alloantigens present in the recipient. Other risk factors that appear to predispose to TA-GVHD in immunocompetent patients possibly include fresh blood, donation from blood-related donors, and Japanese heritage, although the latter two factors probably reflect the HLA homozygous donor. Many affected patients received transfusions of freshly donated cellular blood products. Fresh blood contains larger numbers of viable and presumably competent lymphocytes than stored blood. The minimum number of viable donor lymphocytes required to mediate TA-GVHD is unknown. The one-way match more likely occurs when an HLA haplotype is shared by a donor and a recipient (HLA haploidentical), such as in directed donation from blood relatives and among populations with relatively homogeneous HLA phenotypes. The latter possibility may account for the observation that more cases of TA-GVHD have been reported among Japanese patients.

The clinical features of TA-GVHD are similar to those of acute GVHD following a hematopoietic stem cell transplant, i.e. fever, rash, diarrhea, and liver dysfunction. TA-GVHD is further characterized by prominent pancytopenia due to marrow aplasia. Demonstration of donor-derived lymphocytes in the circulation of a patient with characteristic clinical findings is diagnostic for TA-GVHD. The persistence of donor lymphocytes can be tested by molecular HLA typing, by cytogenetic analysis if donor and patient are of different sexes, and by other molecular polymorphisms. The demonstration of donor-derived lymphohematopoietic cells in a transfusion recipient is not diagnostic of TA-GVHD per se, because donor lymphocytes can be normally detected in the recipient’s circulation a few days after transfusion.

The primary emphasis in TA-GVHD is prevention. Gamma irradiation of cellular blood products with 25 Gy is the effective way of inactivating donor lymphocytes. Irradiation is indicated for susceptible patients with various conditions—e.g. congenital immunodeficiencies, hematopoietic stem cell transplants (both allogeneic and autologous), hematologic malignancies undergoing chemotherapy — and for patients receiving intruterine transfusion, HLA-matched platelets, or blood components donated from a blood relative. Solid organ transplant recipients under immunosuppressive therapy and patients undergoing chemotherapy and radiation therapy for solid tumors probably do not require irradiated blood products. GVHD can occur following transplantation of solid organs, but this is more likely mediated by passenger T lymphocytes present in the transplanted organ than by blood transfusions. TA-GVHD has not been observed in patients with acquired immunodeficiency syndrome.

**Febrile Nonhemolytic Transfusion Reaction**

Febrile nonhemolytic transfusion reaction (FNHTR) is defined as a temperature rise of more than 1°C during or shortly after the transfusion. Fever can be accompanied by chills, and chills in the absence of fever can be considered as a mild febrile reaction. Fever and chills are the most common transfusion reactions, observed in up to 5 percent of transfused patients.

FNHTR is caused either by an interaction between the recipient’s antileukocyte antibodies (usually anti-HLA and less commonly neutrophil-specific) and donor leukocytes contained in the blood components or by pyrogenic cytokines present in the blood components. Alloimmunization to leukocytes occurs commonly in previously pregnant or multiply transfused patients, and they are at higher risk of developing the reaction. Febrile reactions to platelet transfusions may be associated with alloimmunization and poor post-transfusion platelet recoveries. FNHTR is more frequently associated with transfusions of platelets stored for more than 3 days. A number of cytokines—TNF-α, IL-1β, IL-6, and IL-8—are released from leukocytes during the storage of platelets at room temperature, and these cytokines have pyrogenic effects.

Leukoreduction of stored platelets at the time of transfusion is not effective to prevent a febrile reaction, but prestorage leukoreduced platelets have reduced cytokine release during storage and less frequently cause FNHTR. Fresh platelet products with lower amounts of pyrogenic cytokines may be preferred for patients with repeated febrile reactions.

**Transfusion-Related Acute Lung Injury**

Transfusion-related acute lung injury (TRALI) is a rare complication of transfusion resulting in noncardio-
genic pulmonary edema. TRALI is characterized by acute respiratory distress, bilateral pulmonary edema, and severe hypoxemia. Fever and hypotension may be present. Chest X-ray reveals bilateral pulmonary infiltrates.

TRALI is caused by antibodies against HLA (both class I and class II) or granulocyte-specific antigens. Implicated antibodies are usually found in the plasma of transfused blood components. Intravenous immune globulin has also been implicated with TRALI. The antigen-antibody reaction probably activates complement, resulting in neutrophil aggregation and sequestration in the lung. The release of neutrophil granules leads to pulmonary vascular damage and extravasation of fluid into the alveoli and interstitium. Demonstration of the HLA or granulocyte specificity of the donor’s antibody against the patient’s HLA or granulocyte antigen is direct laboratory evidence for TRALI. Less commonly, implicated antibodies are found in the recipient.

Alternative pathogenesis of TRALI includes the possible role of biologically active lipids produced during blood storage. Leukocyte reduction can be helpful in preventing repeat TRALI reactions when the recipient’s antibodies were responsible, but it is not helpful if the antibodies were from the blood donor. All blood donors implicated with a TRALI case should be deferred from the preparation of plasma-containing blood products. Solvent-detergent treated plasma has not been associated with TRALI.

**Neonatal Alloimmune Thrombocytopenia**

Neonatal alloimmune thrombocytopenia (NAIT) develops as a result of maternal sensitization to paternally inherited platelet antigens in the fetus. Antiplatelet IgG antibodies cross the placenta and cause fetal and neonatal immune thrombocytopenia. About half of cases involve the first child. The most commonly involved platelet antigen is HPA-1a (P[1A]). Platelet-specific antigens are generally weak immunogens, and genetic factors may influence whether HPA-1a-negative women will develop anti-HPA-1a antibody. Individuals with certain HLA haplotypes with HLA-DRB3*0101 allele are more likely to develop antibodies against HPA-1a antigen.

Traditionally, it has been thought that NAIT was caused only by antibodies against platelet-specific antigens. However, several case reports suggest that HLA class I antibodies may occasionally be involved.

**HLA AND DISEASE ASSOCIATION**

Certain diseases, especially of autoimmune nature, are associated more frequently with particular HLA types. The association level, however, varies among diseases and there is generally a lack of a strong concordance between the HLA phenotype and the disease. The exact mechanisms underlying the HLA-disease association are not well known, and other genetic and environmental factors may play roles as well.

Among the most prominent associations are ankylosing spondylitis with HLA-B27, narcolepsy with HLA-DQB1*0602/HLA-DRB1*1501, and celiac disease with HLA-DQB1*02. The HLA-A1, B8, DR17 haplotype is frequently found in autoimmune disorders. Rheumatoid arthritis is associated with a particular sequence of the amino acid positions 66 to 75 in the DRβ1 chain that is common to the major subtypes of DR4 and DR1. Type I diabetes mellitus is associated with DR3, 4 heterozygotes, and the absence of asparagine at position 57 on the DQβ1 chain appears to render susceptibility to this disease.

**HLA AND PARENTAGE TESTING**

In paternity testing, genetic markers of a child, biological mother, and alleged father are compared to determine exclusion or nonexclusion of the alleged father.

There are some advantages of using HLA types in parentage testing. The HLA system is inherited in a mendelian manner and is extensively polymorphic; its recombination rate is low; mutation has not been observed in family studies; and antigen frequencies are known for many different ethnic groups. The HLA system, however, does not provide a high exclusion probability when the case involves a paternal HLA haplotype that is common in the particular ethnic group. Molecular techniques using non-HLA genetic systems are widely used, and there is decreasing use of HLA typing for paternity testing.

**SUMMARY**

The human major histocompatibility complex HLA is located on the short arm of chromosome 6. It is
known to be the most polymorphic genetic system in humans. The biological role of the HLA class I and class II molecules is to present processed peptide antigens and thus determine the immune response. The HLA system is clinically important as transplantation antigens. Molecular HLA allele typing is routine to provide HLA class I and class II allele matching in unrelated donor hematopoietic stem cell transplantation. Prospective lymphocyte cross-matching is critical in solid organ transplantation to prevent allograft rejection. HLA alloimmunization causes various problems in transfusion therapy. The HLA system is associated with certain diseases, but its underlying mechanisms are not fully explained.

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Irradiating cellular blood components prevents transfusion-associated graft-versus-host-disease (TA-GVHD) in recipients at risk. Although uncommon, TA-GVHD is usually fatal within 3 weeks of occurrence. Because TA-GVHD may be difficult to diagnose and difficult, if not impossible, to treat, it is most important to try to prevent its occurrence. Since treating cellular components with X-rays or gamma rays can prevent this complication, why not irradiate all components that pose this risk? Simply put, irradiation is not without drawbacks, both economically and with respect to effects on the irradiated component. This chapter assists the reader in establishing preventive policies and procedures for TA-GVHD appropriate for his or her own institution.

HISTORY OF TA-GVHD

Although blood has been transfused regularly for almost one hundred years, TA-GVHD has only recently been fully recognized as a transfusion complication. This is both because of its relatively infrequent occurrence and acquired conditions that place one at greatest risk for TA-GVHD, such as very immunosuppressive chemotherapy or bone marrow transplant have been more common since the 1970s.

In 1955, Shimoda reported a patient with “post-operative erythroderma.” This clinical syndrome is now known to be a form of TA-GVHD. The initial report that helped define TA-GVHD as a unique entity documented erythroderma, hepatomegaly, and fatal aplastic anemia in two infants treated with multiple transfusions of “fresh” blood. Simonsen defined the required elements for a graft-versus-host (GVH) reaction to include: (i) immunologically competent donor cells, (ii) an antigenic difference between graft and host detectable by the donor cells, and (iii) an inability of the host to reject the graft effectively. Subsequently, TA-GVHD was recognized in patients with acquired immunosuppression from intensive chemotherapy or marrow transplantation. Later, the disease was observed in immunocompetent transfusion recipients, who were demonstrated to be at risk of TA-GVHD if the donor was homozygous for the human leukocyte antigen (HLA) antigens for which the recipient was heterozygous.

CLINICAL MANIFESTATIONS

To develop TA-GVHD, donor T cells must recognize immunologic disparity. Many clinical manifestations result from cytokine dysregulation including overproduction of interleukin (IL)-1, IL-2, γ-interferon (IF), and tumor necrosis factor (TNF). The characteristic signs and symptoms of TA-GVHD typically begin 2 to 50 days after transfusion include rash, diarrhea, fever, and elevated bilirubin and hepatic enzymes. An intense pancytopenia distinguishes TA-GVHD from the GVHD that may occur following allogeneic hematopoietic stem cell transplantation. In this latter process, the hematopoietic tissue is derived from the donor and is spared from attack by donor-derived lymphocytes.

The frequent occurrence in Japan of a rash in patients following cardiopulmonary bypass surgery earned it the title of cardiac bypass erythroderma. This phenomenon is now recognized as TA-GVHD from the transfusion of fresh blood collected from the patients’ relatives. The rash of TA-GVHD usually begins as a central maculopapular eruption, sub-
sequently spreads to the extremities, and may progress to bullae formation.

TA-GVHD has a reported mortality in excess of 90 percent. Patients usually die of overwhelming infection secondary to pancytopenia. Yet some cases are not fatal, such as that reported by Cohen et al in 1979.

Organs rich in HLA antigens are at greatest risk of attack during GVHD, including the spleen, liver, gastrointestinal tract (GIT), marrow and lymph nodes, and skin. Pathologic examination including skin biopsy reveals aggressive lymphocytic infiltration.

GENETIC DOCUMENTATION OF TA-GVHD

Confirming TA-GVHD requires documentation that lymphocytes from the blood component donor are present in the recipient in association with the clinical condition. Various techniques provide confirmation including HLA typing, cytogenetic testing, and genomic amplification. Most conveniently, peripheral blood cells are assayed; however, tissue diagnoses may also be performed. Detection of Y-chromosome regions by polymerase chain reaction (PCR) amplification has been used to document circulating cells responsible for TA-GVHD from male donors in female recipients.

Prior to publication of some large Japanese series, the total number of TA-GVHD cases reported in the world’s literature was fewer than 200. The actual incidence of TA-GVHD is not known; any estimation must be based on retrospective reports of cases. One large series over 17 years observed four cases in 847 patients who received non-irradiated fresh (< 48 hours old) whole blood. Although the disease is usually fatal, there is reason to believe that it has been relatively under-reported. Furthermore, because many patients that develop TA-GVHD are quite ill from their underlying diagnosis, it is undoubtedly under-recognized. A recent retrospective review documents that many cases are not recognized particularly in smaller hospitals or in developing countries. Freestanding irradiators contain radioactive material that continually releases a stream of radioactive particles and rays. Freestanding irradiators may use either cesium (Cs-137) or cobalt (Co-60), but Cs-137 is more common due to its longer half-life (30 vs. 5.2 years) and its higher energy. A longer half-life means that dose adjustments are required less often, and a higher energy requires shorter irradiation time to achieve a fixed dose. To protect those in the vicinity

TREATMENT OF TA-GVHD

Treatment of TA-GVHD with a wide variety of immunosuppressive regimens, including steroids, cytoxan, and antithymocyte globulin including OKT3, has been without obvious benefit. Anecdotal successes have been reported following treatment with each of these, however. Often, the diagnosis of TA-GVHD is not made until severe multiorgan damage has already occurred. While milder cases are increasingly being recognized, and recovery from TA-GVHD has been reported, it is not clear that a specific therapeutic regimen has been particularly responsible for recovery from these less aggressive versions of TA-GVHD. In fact, a recent editorial points out that since a wide variety of attempted therapies have been tried and not found to be successful that prevention is paramount.

IRRADIATION OF BLOOD COMPONENTS AS A PREVENTIVE STEP

High energy irradiation produces chemical cross-links within the deoxyribonucleic acid (DNA) of the irradiated cell. The dose is intended to be high enough to damage the DNA but not less sensitive parts of the cell leaving normal cellular functions unaffected but preventing cellular reproduction. Since TA-GVHD requires cellular proliferation of the donor lymphocytes, prevention of cell division effectively precludes a significant GVH response.

Although irradiation may cause malignant transformation, available data indicate that this risk must be very small.

Types of Irradiator Units

Freestanding irradiators contain radioactive material that continually releases a stream of radioactive particles and rays. Freestanding irradiators may use either cesium (Cs-137) or cobalt (Co-60), but Cs-137 is more common due to its longer half-life (30 vs. 5.2 years) and its higher energy. A longer half-life means that dose adjustments are required less often, and a higher energy requires shorter irradiation time to achieve a fixed dose. To protect those in the vicinity
Handbook of Blood Banking and Transfusion Medicine

of the irradiators, the source is massively shielded by lead, resulting in both extremely expensive (>US$200,000 (United States $)/unit] and heavy (2–3 ton) devices. Alternatively, X-ray irradiator units do not have a radioactive source and hence are cheaper and smaller, but provide less capacity/batch irradiated. In addition, costs of maintaining freestanding units include licensing, quality control, and safety monitoring.

Linear accelerators can also be used. For centers without access to a freestanding irradiator but having a radiation therapy center available, the components may be irradiated using a linear accelerator. While this method results in an equivalent component, the logistics of calling in a team to irradiate after hours or on weekends, decreases the practicality of this option.

Dosage of Irradiation to Prevent TA-GVHD

The optimal dose for TA-GVHD prophylaxis is unknown. Doses as low as 5 gray (Gy) significantly attenuate (2/3rd decrease) tritium (3H) thymidine uptake in mixed lymphocyte culture assays. However, recipients of components irradiated in devices where the intended midplane dose was as high as 15 and 20 Gy have acquired TA-GVHD. Consequently, the US Food and Drug Administration (FDA) has suggested a midplane dose of 25 Gy with the minimum dose of 15 Gy to any point within the container. The American Association of Blood Banks (AABB) Standards require the intended dose of irradiation to be at least 25 Gy delivered to the midplane of the canister if a freestanding irradiator is used or to the central midplane of the field if a radiotherapy instrument is used. The minimum dose at any point in the canister or field must be 15 Gy. There have been no reports of TA-GVHD when components have received this dose. Pelszynski et al used a sensitive in vitro assay for T-cell proliferation and concluded that a gamma-irradiation dose of 25 Gy may be required to inactivate completely the T cells in units of red blood cells (RBCs). Currently, most centers irradiate to an intended dose of 25 Gy to the midplane.

The FDA has stated that the maximum irradiation dose should be 50 Gy. Thus, a dose of 25 Gy would permit a blood component to be irradiated up to twice and released for transfusion. In contrast, if a higher dose were used, a component irradiated twice could not be transfused.

Adverse Effects of Irradiation on Blood Components and Storage

The 24-hour post-transfusion recovery of irradiated red cells, after storage, is decreased compared with that of nonirradiated cells. Red cells irradiated within 24 hours of collection maintain acceptable viability for up to 28 days. The FDA requires that irradiated RBCs not be stored for more than 28 days from the date of irradiation but also not more than the dating period of the nonirradiated product.

Significant changes in platelet function have not been found in irradiated platelets and stored for 5 days. No change in storage time is necessary for platelet concentrates.

Although high irradiation doses may impair cell function, one study suggests the lack of any adverse consequences to red cells, platelets, or granulocytes up to 50 Gy. A 1997 report observed lipid peroxidation and hemoglobin oxidation at clinically used doses, but the clinical significance of these findings is unclear.

Irradiated units of RBCs contain approximately twice as much extracellular potassium as unirradiated units of similar age as well as greater levels of cell-free hemoglobin. The clinical significance of increased potassium is most significant if the transfusion is intended for a neonate or infant. Many pediatric institutions try to use irradiated units within 48 hours or immediately after irradiation if intended for exchange transfusions to neonates.

Jin et al demonstrated that gamma irradiation of RBCs from donors with sickle cell trait did not result in any differences in plasma potassium or red cell adenosine triphosphate (ATP) compared with units from donors with hemoglobin AA. Miraglia et al showed that autologous 24-hour post-transfusion recoveries of frozen irradiated red cells were acceptable.

The increasingly global distribution of blood components raises the practical question of whether there are any adverse effects from the irradiation dose used by conventional airport security scanners. The average dose is 5 logs lower than the 25 Gy to which
blood is exposed for prevention of TA-GVHD. Petzer et al. calculated that marrow being flown to Europe during a 10-hour flight gets exposed to 40 times the irradiation dose of an airport scan, simply from decreased atmospheric protection. Following the events of 9/11/2001, it is permissible to use airport metal detectors to scan hematopoietic progenitors during transport. Specifically, the AABB hematopoietic standard 9.5.1.4 now reads: “The HPC therapy service shall ensure that products are not exposed to gamma irradiation, inappropriate temperatures, or other damaging conditions that could harm viability, recovery or sterility.”

Quality Control and Quality Assurance

In the United States, the federal government provides guidelines regulating the dosage and administration of radiation to blood cells. These are largely consistent with European Union (EU) guidelines as well. The 2000 FDA memorandum regarding blood product irradiation states that the dose of irradiation delivered should be 25 Gy targeted to the central portion of the container and that 15 Gy should be the minimum dose at any other point. Validation studies should be performed to establish that the irradiator performs within the above limits and maintenance procedures should ensure that satisfactory performance continues. The validation studies should be performed annually and after mechanical repairs, and the use of indicator devices to signal exposure of the product to irradiation is recommended.

The AABB Standard 5.7.4.2 requires that “an indicator be used with each batch of components that are irradiated.” Irradiation indicators should be considered qualitative. Since there is a considerable range in dosing throughout an irradiation canister (possibly up to 25 percent attenuation of the beam as it passes through an aqueous medium), it is most practical to use indicators that require only the minimum acceptable dose (i.e. 15 Gy), to avoid being faced with the quandary of an incompletely changed indicator on a unit from the bottom of the canister.

The AABB Standards further require that when any component has been irradiated, it should be so labeled. As noted, the expiration date of irradiated RBCs needs to be changed not to exceed 28 days if more than this time remains until the original day of outdate. Training of those individuals performing irradiation must include safety procedures and be appropriately documented. The irradiator must be monitored for leakage.

General Indications for Irradiation of Blood Components

TA-GVHD is fundamentally an immunologic battle between the invading transfused T cells and the host’s immune defense. Indeed, Lee et al. have demonstrated the existence of an in vivo mixed lymphocyte reaction that occurs following transfusion of leukocytes into a normal host. Specifically, the number of donor leukocytes rapidly decreases as the cells are cleared from the circulation over several days, followed on days 3 to 5 by a 1-log expansion of donor T lymphocytes. In the normal setting, host immune mechanisms remove the remaining cells; in some cases, it is possible to demonstrate long-term stable chimeras. In military terms, the likely winner of the skirmish between donor and host immune cells is determined by multiple factors, including the relative number of troops on each side (i.e. the number of donor vs host leukocytes), the competitiveness of each combatant (the immune competence of the donor and host), and the fatigue of each army (fresh vs stored donor cells; previous host exposure to immunosuppressive irradiation and/or chemotherapy disease). Hence, increased risk of TA-GVHD is associated with higher numbers of transfused lymphocytes (e.g. exchange transfusions in premature infants or granulocyte transfusions) or an immunosuppressed host (congenital or acquired).

An exception to TA-GVHD requiring an immune compromised host is when the donor cells appear immunologically (HLA) identical to the cells of the recipient, but the recipient’s cells appear foreign to the donor. This may result when a donor is homozygous for a given HLA haplotype for which the recipient is heterozygous, and it occurs most commonly (i) with a relatively high-frequency HLA haplotype in a given population (e.g. Japan), and (ii) following related donor transfusions. This situation is discussed below.

Which components may cause TA-GVHD?

Cytotoxic T cells mediate TA-GVHD. Hence, any component that contains sufficient, viable cytotoxic T cells can, in theory, mediate TA-GVHD. In practice, only cellular components contain sufficient viable cells...
to be of concern. While there have been reported cases of TA-GVHD following fresh plasma infusion,37 no cases have been reported following administration of fresh frozen plasma (FFP). Other components known to have caused TA-GVHD include whole blood (fresh and stored), RBCs, granulocytes, and platelet concentrates.38 Frozen components, including frozen washed red cells, FFP, and cryoprecipitate, have not been associated with TA-GVHD.

INDICATIONS FOR IRRADIATING CELLULAR BLOOD COMPONENTS

Table 9.1 details indications for receiving irradiated cellular components. These are divided into categories of absolute indications, probable indications, controversial indications, and indications where irradiated products are not required. What follows is a discussion of selected indications from each of the categories.

Absolute Indications

**Congenital Cellular Immunodeficiency**

Many of the earliest reports of TA-GVHD described infants and children with congenital disorders of cellular immunity who developed complications following blood transfusion.2 Early reports included TA-GVHD in severe combined immunodeficiency patients,39 thymic hypoplasia,40 and Wiskott-Aldrich syndrome.41 Because all three of these immune deficiencies are rare, there is often a significant delay before a diagnosis is definitively made. For example, treatment for some severe infectious complication may require transfusion support before irradiation of blood components is implemented. In addition, thymic hypoplasia may occur in the setting of congenital cardiac anomalies, (i.e. DiGeorge syndrome), making it likely that the child would require cardiac surgery. Hence, many pediatric cardiac surgeons require that all infants with congenital cardiac aortic arch defects or intracardiac anomalies (e.g. tetralogy of Fallot, truncus arteriosus, pulmonary and aortic valve defects) receive irradiated blood until the chromosomal defect associated with DiGeorge syndrome is excluded. Congenital immunodeficiencies as parts of rare syndromes may be difficult to recognize. For example, a case of TA-GVHD was reported in a patient with multiple intestinal atresias, now known to be associated with a congenital immunodeficiency.42

**Hematopoietic Stem Cell Transplant Recipients**

Both allogeneic and autologous recipients of hematopoietic stem cell transplants have developed TA-GVHD.63,44 Total body irradiation appears to put the recipient at particular risk. It is universal practice to provide irradiated cellular components during hematopoietic stem cell transplants. More controversial is whether these recipients should continue to

| Table 9.1: Indications for irradiation of cellular blood components for prevention of transfusion-associated graft-versus-host disease |
| Absolute indications |
| • Patients with congenital cellular immune deficiency2,39-42 |
| • Allogeneic hematopoietic stem cell recipients63 |
| • Autologous hematopoietic stem cell recipients53 |
| • Patients with Hodgkin’s disease50,51 |
| • Granulocyte transfusions52 |
| • Intrauterine transfusions (IUT)53-54 |
| • Transfusions to neonates who have received IUT53-54 |
| • Transfusions from biologic relatives5,13,57,58 |
| Probable indications |
| • Premature neonates < 1500 g60-62 |
| • Patients with hematologic malignancies (other than Hodgkin’s disease) treated with cytotoxic agents4,65 |
| • HLA matched and/or crossmatched compatible platelet concentrate transfusions68,69 |
| • Patients receiving high-dose chemotherapy, radiation therapy, and/or aggressive immunotherapy10,66-67,71 |
| Controversial indications |
| • Solid organ transplant recipients76-77 |
| • Large volume transfusions and exchange transfusions to term neonates who did not receive IUT |
| • Aplastic anemia patients not receiving aggressive immunotherapy70 |
| • Term neonates on ECMO79 |
| Irradiation not indicated |
| • Patients infected with the human immunodeficiency virus (HIV) |
| • Patients with hemophilia |
| • Small-volume transfusions to term neonates who did not receive intrauterine transfusions |
| • Elderly patients |
| • Patients receiving immunosuppressive medications (see text) |
| • Immunocompetent surgical patients |
| • Pregnant patients |
| • Patients with membrane, metabolic, or hemoglobin red blood cell disorders (e.g. thalassemia, sickle cell disease) |

(Adapted from Table 17-1 in Transfusion-Associated Graft-versus-Host Disease Jed B. Gorlin and Paul D. Mintz in Transfusion Therapy: Clinical Principles and Practice, AABB Press 1999, Bethesda, with permission)
require irradiated products on subsequent admissions. In practice, most centers leave the requirement for special components (e.g., leukocyte-reduced, irradiated) as part of the patient requirements for a year and in some cases indefinitely since immunocompetence may not be fully restored following recovery.

Increasingly, allogeneic hematopoietic stem cell transplant recipients are candidates for donor lymphocyte infusions (DLI) as a way of improving engraftment or treating relapse.45 Alas, these infusions come with the price of increased risk of GVHD. Hence, the more CD3 positive cells/infusion and type of relapse were predictive of worse GVHD.46 Partially depleting the DLI of CD8+ cells seems to decrease the extent of GVHD.47 Since this form of TA-GVHD overlaps the traditional and expected risk of GVHD from allogeneic transplants, it will not be extensively reviewed here.

Co-administration of donor leukocytes with solid organ transplants has been used to induce tolerance to donor organs.48 Unfortunately, co-administration of donor leukocytes is not without the hazard of inducing TAGVHD and fatal results have been observed.49

Hodgkin’s Disease

There are many reports of TA-GVHD in patients with Hodgkin’s disease.50 This may follow from an underlying defect in immune regulation. Therefore, irradiation of cellular components for patients with Hodgkin’s disease is considered an absolute indication.51

Granulocyte Transfusions

Granulocyte transfusions involve large numbers of very fresh leukocytes (including lymphocytes) being infused into immunologically compromised hosts; consequently, multiple reports associate this component with TA-GVHD.52 Thus, granulocyte concentrates must be irradiated prior to transfusion.

Intrauterine Transfusions and Transfusions to Neonates who have Received Intrauterine Transfusions

All intrauterine transfusions must be irradiated. In 1969, Naiman et al53 reported a case of fatal TA-GVHD in an 8-week-old infant who had received three intrauterine transfusions, one of which was implicated by cytogenetic studies. In 1974, Parkman et al54 reported fatal TA-GVHD in two infants after intrauterine transfusion and exchange transfusion. In both of these patients, the lymphocytes causing the GVHD were demonstrated to have come from an exchange transfusion donor. The authors commented that in the preceding 27 years, no infants treated with exchange transfusion alone had fatal TA-GVHD. They speculated that the introduction of lymphocytes by intrauterine transfusion had rendered the infants susceptible to TA-GVHD from the exchange transfusion. They suggested that irradiating blood used for exchange transfusion would be appropriate in this circumstance. In practice, irradiation of all cellular components transfused to neonates and infants who have received intrauterine transfusions is recommended.

Many transfusion services simply find it practical to provide irradiated blood for all neonatal exchange transfusions.

Transfusions from Biologic Relatives

TA-GVHD occurs most frequently in immunocompetent recipients. Most of the patients received blood from a donor homozygous for an HLA haplotype for which the recipient was heterozygous (either HLA-A and -B or HLA-A,-B, and -DR). This situation, first reported in detail by Thaler et al,5 allows the donor cells to react against the foreign recipient, while the host’s immune system views the donor lymphocytes as self and does not reject them. Previously, the syndrome “postoperative erythroderma” was recognized in Japan among patients who had received fresh blood typically from relatives.1 In a subsequent review of TA-GVHD among immunocompetent Japanese recipients, the majority of cases (62%) occurred with the use of fresh blood (< 72 hours from donation) but in only a minority of cases (29%) were the donor and recipient related.13 Among donors and recipients who were studied, in 93 percent of cases, the donor was HLA homozygous for a haplotype shared with the recipient. Petz et al55 comprehensively reviewed the English-language literature regarding TA-GVHD in immunocompetent recipients. They noted the use of fresh blood (<96 hours from donation) in 87 percent
of cases, a family relationship between donor and recipient in 44 percent, and an HLA homozygous donor for a haplotype shared by the recipient in 87 percent of cases. An interesting case report describes the quandary of an allogeneic-related transplant where the donor was known to have a homozygous for HLA type. The donor peripheral blood stem cells were CD34 concentrated and selected add back of donor T cells were administered. The patient successfully engrafted without excessive GVHD.56

Kanter57 has calculated that the probability of this situation existing among relatives is greatest for transfusions between parents and children, next most likely for transfusions between grandparents and grandchildren or between aunts/uncles and nieces/nephews, and third most likely among siblings.57 Wagner and Flegel58 calculated that the risks for this occurrence between parents and children is increased at least 21-fold for US Whites, 18-fold for Germans, and 11-fold for Japanese compared with the risks from nonrelated transfusions.

In the United States, cellular blood components from all biological relatives are irradiated. Many hospitals will irradiate blood from all directed donors because eliciting whether a directed donor is related by blood to a particular recipient is fraught with uncertainty.

The widespread use of related (“replacement”) donors in developing countries greatly exacerbates this problem, especially since many developing world’s transfusion services do not have the resources to obtain and maintain devices for irradiation.14 The WHO push towards centralized transfusion services in the developing world may have the side benefit of helping to decrease the small but serious risk of this complication.59

**Probable Indications**

**Transfusions to Premature Infants Weighing Less than 1,500 g**

Whether transfusions to premature infants require irradiation has been the subject of conflicting opinions. Many centers have established policies to ensure that extremely premature infants receive irradiated blood. Specifically, in a neonatal transfusion practices survey, a majority of the responding institutions reported that they provide irradiation for at least some neonates.60 Many institutions choose to irradiate all cellular components dispensed to their own neonatal intensive care unit. However, it is questionable whether gamma irradiation of blood components is needed for neonates other than those in the recognized high-risk groups of congenital cellular immunodeficiency diseases, recipients of intrauterine transfusions with or without subsequent exchange transfusion, and recipients of blood components from blood relatives. Most case reports of TA-GVHD in premature infants have had additional confounding risk factors, including underlying immunodeficiencies, related donors, and/or prior intrauterine transfusions. Only a few reports document TA-GVHD in a premature infant without other risk factors from an HLA-desperate donor.60

Nevertheless, premature infants represent one of the most frequently reported groups at risk for TA-GVHD.61,62 In addition, premature infants weighing less than 1,500 g have relatively poorly developed cell-mediated immunity. In fact, the empirical observation is that premature infants with TA-GVHD without confounding risk factors were usually under 1,500 g at birth.61 While it must be emphasized that these case reports represent exceptions to the great number of uneventfully transfused premature infants, in aggregate they represent a substantial portion of the total case reports of TA-GVHD.53 Therefore, most strongly recommend irradiation of cellular components for extremely premature infants (< 1,500 g) as a prudent precaution. However, the routine irradiation of cellular blood products for all neonates is not recommended.64

**Patients with Hematologic Malignancies (other than Hodgkin’s disease) Treated with Cytotoxic Agents**

Irradiation of cellular components for patients with non-Hodgkin’s lymphoma and leukemias is often categorized as a probable indication; that is, multiple cases have been reported, but it is not yet universal practice to provide irradiated components for these patients.4,65 In short, the risk for TA-GVHD is less in patients with hematologic malignancies other than Hodgkin’s disease, but still well reported.
Transfusion-associated Graft-versus-Host Disease

Patients Receiving High-Dose Chemotherapy, Radiation Therapy, and/or Aggressive Immunosuppressive Therapy

Transfusion following aggressive chemotherapy for other malignancies, especially neuroblastoma, has been associated with TA-GVHD.66 Patients with rhabdomyosarcoma,67 glioblastoma,52 and renal adenocarcinoma68 have also reportedly developed TA-GVHD. The glioblastoma case followed a granulocyte transfusion, which, as discussed above, is now considered an absolute indication for irradiation. Thus, irradiated cellular blood components are recommended for patients with malignancies who are receiving therapy that may cause severe immune deficiency. Routine irradiation of blood products is not indicated for patients with solid tumors in the absence of intensive immunosuppressive therapy.69

Because TA-GVHD has been reported in patients with aplastic anemia,70 it is appropriate to irradiate cellular products for such patients who are receiving intensive immunosuppressive regimens. A recent fatal case of GVHD is a patient with a nonmalignant disease, lupus erythematos is treated with fludarabine, underscores the observation that the risk is due to the degree of immunosuppression and not necessarily the underlying disease.71 Hence, there are increasingly calls for a lower threshold to require irradiation.20 In short, any highly immunosuppressive regimen appears to increase the risk of TA-GVHD.

Platelet Donors Chosen for HLA Matching or Crossmatch Compatibility

All platelet concentrates selected on the basis of crossmatch compatibility and/or HLA matching should be irradiated. Donors homozygous for HLA haplotypes that match the recipient may be over-represented among those selected as HLA matches for recipients, because such homozygous donors will express fewer HLA antigens and will be more likely not to express antigens found in the recipient. Grishaber et al72 noted that in their experience with patients who received platelets matched for Class I HLA antigens, in 5 percent of transfusions, the recipient received lymphocytes from a donor exhibiting no foreign antigens, but the patient had antigens not present on donor lymphocytes. Twenty-three percent of patients received at least one such transfusion. Takahashi et al73 calculated that the risk of receiving a one-way HLA-matched blood transfusion from an unrelated donor in the United States among Caucasians is 1 in 797 and in Japan, 1 in 312.

TA-GVHD has been reported in recipients of unirradiated HLA-matched platelets from unrelated donors, but such cases are relatively rare.74 This may be because the process is not recognized, because minor histocompatibility incompatibilities may be more likely to exist among unrelated recipients and could lead to donor lymphocyte clearance, or because idiotypic antibody to the T-cell receptor is present in multitransfused patients.75

Controversial Indications

Solid Organ Transplantation

Immunosuppressive regimens following solid organ transplantation are historically not as aggressive as those associated with hematopoietic stem cell transplantation. Nonetheless, especially with advances in immunosuppression for liver transplantation, the regimens have grown more potent. TA-GVHD following liver transplantation, possibly associated with underlying host cytopenias, has been reported in cases documented to have mutually discordant HLA types.76,77 Nonetheless, it is not routine practice to irradiate blood for solid organ allograft recipients.

Aplastic Anemia Patients not on Aggressive Immunosuppressive Therapy

TA-GVHD has occurred following treatment for aplastic anemia.78 As noted, the authors do not believe that patients with this disease require irradiated cellular blood components unless they are receiving aggressive immunosuppressive therapy.

Term Neonates Undergoing Extracorporeal Membrane Oxygenation

While thousands of neonates have been treated using extracorporeal membrane oxygenation and have required massive transfusion support using blood components that were not irradiated, TA-GVHD is exceedingly rare in these patients. Nonetheless, there is at least one published case of TA-GVHD in a full-term infant treated for neonatal respiratory distress
following meconium aspiration. On day 17, the child developed a florid rash and fever. Skin biopsy was consistent with GVHD. Despite treatment with steroids and cyclosporine, multiorgan failure ensued and the child died. No underlying immune defect could be demonstrated including documentation of normal number of T cells and mitogen stimulation studies. The specific donor mediating the TA-GVHD was not identified; hence, the possibility of a one-way HLA match by chance could not be ruled out in this case.

Nonindications

**Human Immunodeficiency Virus Infection**

No case of TA-GVHD has occurred following acquisition of human immunodeficiency virus (HIV) infection despite the numerous unirradiated transfusions to which this patient group has undoubtedly been exposed, which illustrates that the risk factors for TA-GVHD are more complex than simple immunodeficiency. Since the primary immunodeficiency of acquired immune deficiency syndrome is in the CD4+ T cells, it might be posited that the cytotoxic T cells responsible for host defense against TA-GVHD remain intact. An animal model confirms the importance of CD8+, as opposed to CD4+, cells being critical for host defense against TA-GVHD. Indeed, it took far more donor cells to cause GVHD when CD4+ cells were depleted in the host than when host CD8+ cells were depleted. The Viral Activation Transfusion Study Group (VATS) examined whether there was significant donor chimerism in HIV patients who received transfusions. Half the group received non-leukoreduced units, and hence, there was the opportunity to assess the natural history of donor cells following transfusion. No subject had donor cells detectable more than 4 weeks after transfusion, similar to other transfused subjects studied. Alternatively, HIV infection of donor CD4+ cells could prevent them from mounting GVHD.

**Small-Volume Transfusions to Term Neonates who did not Receive Intrauterine Transfusion**

With the exception of the child treated with extracorporeal membrane oxidation described above, there are no reports of full-term neonates without other risk factors developing TA-GVHD. Term neonates do not routinely require irradiated cellular components. As noted, some programs will irradiate all exchange transfusions to term neonates rather than attempt to rule out who has received an intrauterine transfusion.

**Elderly Patients**

Elderly recipients do not appear to be at increased risk of TA-GVHD solely on the basis of age. Since a considerable proportion of blood transfused is received by patients aged 60 or older, it must be presumed that an adequate opportunity exists to detect a significantly increased risk. Hence, blood should not be irradiated solely because the recipient is elderly.

**Patients Receiving Immunosuppressive Medications**

Patients with autoimmune diseases who have received conventional immunosuppressive medications have not been reported to have developed TA-GVHD. Therefore, irradiation of cellular blood products for these patients is not indicated. However, the recent case of TA-GVHD in a lupus patient following fludarabine and the use of stem cell transplants in various forms of autoimmune disease emphasize that any recipient of strong immunosuppressive therapy requires irradiated blood components.

**POSSIBLE ALTERNATIVES TO IRRADIATION**

**Leukocyte Reduction**

In theory, since the risk of TA-GVHD is a function of the dose of infused immunocompetent T cells, the effective removal of these cells should prevent this complication. In practice, the use of leukocyte reduction for prophylaxis against TA-GVHD is precluded by the variable efficiency observed in leukocyte reduction with current technology and the variable number of leukocytes required to mediate this complication. Indeed, documented cases of TA-GVHD have occurred despite transfusion of solely leukocyte-reduced units to the recipients.

**Ultraviolet Pathogen Inactivation Methods**

Alternatively, while not yet licensed for this application, photochemical inactivation of bacterial and
viral pathogens in blood components using psoralens and ultraviolet (UV)-A light irradiation may also serve as prophylaxis against TA-GVHD. Several reports document the efficacy of this process to inactivate T cells that mediate TA-GVHD using both in vitro and in vivo models.\textsuperscript{84} It has also been shown to prevent TAGVHD from platelets.\textsuperscript{85} Hence, when this process gains acceptance as a method to reduce transfusion-related infectious risks, it may have the side benefit of obviating the need to irradiate products for individuals at risk of TA-GVHD.

Alternatively, simply irradiating leukocyte-containing components with the less intense UV-B light may prevent TA-GVHD. A mouse model documented prevention of GVHD using UV-B irradiated donor leukocytes.\textsuperscript{86} Again, since licensed devices to irradiate components using UV-B light are not available, and since the presence of red cells in the irradiated product complicates the efficacy of this treatment, further advances in this technology are required before it can be routinely applied to prevent TA-GVHD.

CONCLUSION

TA-GVHD is a devastating complication of cellular blood component transfusion. Although TA-GVHD is most often considered in immunocompromised patients, the development of this process in immunocompetent patients who received blood from relatives is a consequence of the demand for directed donations. The relatively low-cost, low-risk procedure of 25 Gy of gamma irradiation has afforded a reliable prevention.

REFERENCES

The infusion of a blood component is a therapeutic procedure that must be performed step-by-step with caution and care. If performed correctly the most common transfusion-related fatal complication, hemolysis due to ABO incompatibility, can be prevented. By careful blood component administration and stopping of transfusions at the early signs of a reaction, the severity of other complications such as volume overload, bacterial sepsis due to contaminated blood and transfusion-related acute lung injury can be lessened.

The greatest single risk associated with blood transfusion is the administration of the wrong blood to the wrong patient resulting in a fatal hemolytic transfusion reaction caused by ABO incompatibility.1-13 Except for two smaller reports of bacterial contamination as being the most frequent cause,14,15 human error causing an ABO incompatible hemolytic transfusion reaction has consistently been the most common cause of transfusion-related death in the US over the last several decades. Most of these deaths were due to misidentification of the patient at the time of transfusion. The second most frequent cause has been the use of a mislabeled blood sample obtained from the wrong patient for blood typing and compatibility testing.16-18

After the physician has ordered a transfusion and the proper blood component has been selected, a set of steps and procedures involving the physicians and nurses at the bedside or during surgery are set into motion and if performed correctly result in a safe transfusion of the correct component and the intended benefit to the patient. Hospitals must have written procedures for obtaining properly labeled blood samples from the patient, for the nursing staff to obtain blood components from the blood bank, for infusing the blood component into the proper recipient and the recognition and early management of transfusion reactions. Each step in the transfusion process should be documented in the patient’s medical record.

The first step in safe transfusion is to order a blood transfusion only when needed. Using blood judiciously can avoid the use of unneeded blood and prevents harm to patients. Physicians should be knowledgeable as to the appropriate use of blood components and the hospital blood bank, blood bank medical director or hospital transfusion committee should have written indications and guidelines for blood component use. Hospitals should also have procedures in place for auditing and reviewing transfusions for appropriateness and comparing blood use to established criteria and written guidelines. Ideally, the review of transfusion appropriateness is a peer review of one physician’s use of blood for a patient reviewed by another physician and reported to the hospital transfusion committee, a committee of the hospital
medical staff, if the transfusion is deemed inappro-priate.

Hospitals must also have written procedures for nurses and other hospital staff for obtaining informed consent for blood transfusion, ordering and obtaining blood from the blood bank, blood administration and the recognition and early management of transfusion reactions. A hospital written procedure regarding patient identification and blood sample labeling should also exist. The development of these written procedures should be a collaborative effort of the transfusing physicians, hospital nursing personnel, hospital blood bank and laboratory personnel and the blood bank medical director. With these procedures in place, the multistep process of blood transfusion has the greatest likelihood for a safe outcome and intended benefit for each patient.

PHYSICIAN’S ORDER AND INFORMED CONSENT

Blood component administration begins with an order by the physician. Following the clinical assessment by the physician that a transfusion is indicated, a written order is placed in the patient’s medical record for a patient blood sample for ABO, Rhese (Rh) type and compatibility testing (blood type and antibody screen or blood type and crossmatch); type of and number of components to transfuse, i.e. 2 units of red cells, etc.; special requirements, if any, such as gamma irradiation, volume reduction, washing or leukoreduction by filtration; when the component is to be started; the rate of infusion; and premedications such as antipyretics or antihistamines, if needed. It is important that the physician’s order be complete, clearly documented and available on the patient care unit for reference by nursing personnel so the person giving the transfusion can review the order immediately prior to blood infusion to confirm that the component to be transfused meets the specifications of the ordering physician.

It is a standard of practice in several countries that the physician obtains informed consent from the patient prior to non-urgent transfusions so that the patient knows of the risks, benefits and alternatives, including a choice to decline a transfusion. Some hospitals require that patients, who receive multiple transfusions, need only give consent once a year when the transfusions are for a single treatment such as a marrow transplant or a single chronic disease and only once per hospital admission when the transfusions are related to another disease or surgical treatment.

PATIENT BLOOD SAMPLE COLLECTION AND LABELING

Proper blood sample collection begins with patient identification. This is the first major step in providing a safe transfusion. A request for laboratory studies, including blood typing, red cell antibody testing and compatibility testing, is generated from the physician order. This request includes patient identification information such as name and medical record number. The hospital policy for patient identification must be followed and generally requires use of two patient-specific identifiers, most often patient’s first and last name and medical record number. Supplies needed for blood collection include the request form, sample labels and the phlebotomy tray with tubes, needles, antiseptic wipes, tourniquet, etc. all of which should be brought to the patient. The patient should be wearing an identifying wristband. The procedure starts with patient identification and confirming that the name and medical record number or other identifier on the wristband are identical to the labels to be placed on the blood tubes at the bedside at the time of or immediately after blood collection. The phlebotomist should ask the patient to audibly state his or her name to confirm that the tube is labeled accurately. Improper patient identification has resulted in several avoidable fatalities due to incompatible blood transfusion. It is the responsibility of the phlebotomist to prove the identity is that of the patient to be drawn before every blood sample collection.

After the blood sample is collected, the label is applied and the phlebotomist adds the date, time and the phlebotomist’s initials to the label. This additional information is required for laboratory reference should questions regarding sample integrity or patient identification arise. The labels are placed on the tubes at the time of phlebotomy and in the presence of the patient, at the bedside, to prevent misidentification errors. Unlabeled patient samples should not be taken to another area, such as the nursing desk, for labeling. It is advisable that sample labeling be performed only by the phlebotomist who identified the patient and confirmed that the request form and labels were accurately identified by the correct patient’s name.
Patient misidentification occurs more frequently when samples are labeled by persons other than the phlebotomist.

LABORATORY TESTING

Red Cell ABO Typing and Compatibility Testing

The labeled patient sample and request are sent to the lab. The blood bank laboratory personnel should first confirm that the request and labeled sample match and that the sample label also has the date, time and initials of the phlebotomist. Should any of this information be missing or not match, including typographical or numbering errors on a hand labeled sample, then the sample is considered mislabeled and discarded and a new sample requested. It is the standard of care in many countries that tests are never performed on a mislabeled blood bank sample. The phlebotomist is contacted and the reason for the labeling error investigated. A program should be established by the blood bank to monitor the rate of misidentified blood samples and implement a corrective action program when needed.19-21 Some hospitals provide written feedback to phlebotomists, nurses and their supervisors whenever a misidentified blood sample is received by the blood bank.

The patient’s ABO and Rh type must be known for transfusion of any blood component. There should be at least two separate typings performed to assure that the proper ABO and Rh is attributed to the patient. This is accomplished from two separate blood samples or a single patient sample can be typed twice for ABO and Rh and this is done by two separate laboratory technicians if possible. When there is a previous blood type in the blood bank hospital record for the patient, the ABO and Rh tests can be performed once and compared to the previous results for accuracy. If blood components are received from a facility such as a blood donor center, the hospital blood bank should confirm that all red cell components in their inventory are of the type described on the label by performing ABO and Rh typing of the components. Discrepant results on patient or unit typing must be fully investigated and documented with corrective action.

Compatibility testing must be performed before red cell transfusions. Other than for ABO and Rh, full compatibility testing is not needed for plasma, cryoprecipitate and platelet transfusions. If red cell antibody testing is performed on patients, any patients with known red cell alloantibodies require antigen negative red cell units fully crossmatched through the antihuman globulin phase. For patients with negative antibody screens, ABO and Rh compatibility with each red cell unit transfused must be assured but an abbreviated method can be used, such as crossmatch at room temperature with immediate centrifugation (immediate spin crossmatch) or by an automated, computerized matching of previous patient ABO typing records (computerized crossmatching) to current ABO typing results and the intended red cell component ABO and Rh type.22

TIMING OF THE PRE-SURGICAL BLOOD GROUP TYPING AND COMPATIBILITY TESTING

In many hospitals, it is common for blood samples to be sent to the blood bank on the same day as surgery.23-25 Ideally, the blood samples are provided to the blood bank several days before scheduled surgery so that surgery is not delayed should it be difficult to identify compatible blood. A 1987 study noted that 2.4 percent of patients admitted on the same day as surgery were found to have red cell antibodies and incompatibilities with red cell components.23 Another study showed that 9 percent of patients had surgery started without compatible blood available because the blood sample had been provided to the blood bank so close to the time of surgery.25 In many hospitals it is permitted to perform blood typing and compatibility testing on a blood sample obtained up to 10 to 14 days prior to surgery or the scheduled transfusion. If the patient has been pregnant or transfused within the previous three months, the blood sample must be obtained within three days of the transfusion so that the antibody testing and compatibility testing reflect the current antibody status of the recipient. The pretransfusion blood sample obtained from the recipient and a sample or segment from red cell components transfused should be stored at 1 to 6°C for at least seven days after the transfusion to be available for use in the investigation of adverse transfusion outcomes.

THAWING AND POOLING COMPONENTS

To prepare frozen plasma or cryoprecipitate for infusion, units are thawed in a water bath at 30 to 37°C
with gentle agitation. It generally takes about 20 minutes to thaw a unit of plasma and some water baths can thaw 4 units simultaneously. If not used immediately, thawed plasma should be stored at 1 to 6°C. Thawed cryoprecipitate, if not immediately used, can be temporarily stored at room temperature, 22 to 24°C, to prevent re-precipitation. Factor VIII and fibrinogen in thawed cryoprecipitates are better preserved at room temperature than at 1 to 6°C.\(^{26}\) If ambient room temperature is 30°C or more thawed plasma should not be stored but should be used immediately after thawing. Cryoprecipitates have an expiration time of 4 hours after pooling. The expiration time after thawing of cryoprecipitates is 6 hours if not pooled. Thawed plasma is not pooled and has an expiration period of 24 hours from the time of thawing if used to treat coagulopathy. Thawed plasma that was not entered or used by the original intended recipient and was returned to the blood bank, may be stored for up to 5 days at 1 to 6°C and used for another patient.\(^{27-29}\)

Because of a reduction of factor VIII levels and milder reduction of factor V levels, plasma stored up to 5 days should not be used for patients with those selective deficiencies but can be used for all other patients.

ABO-identical units of platelets concentrates can be pooled prior to transfusion, using aseptic technique, for ease of bedside administration. The pooled units should be transfused within four hours to prevent the risk of proliferation of any bacteria present. If pooled platelet components are created using a sterile connecting device, the expiration date can be that of the oldest unit in the pool.

**SPECIAL PROCESSING**

Other component processing that may be needed prior to transfusion includes gamma irradiation to prevent transfusion-associated graft-versus-host disease, washing of red cells or platelets to remove plasma for patients with severe reactions to plasma proteins, volume reduction by gravity sedimentation or centrifugation for patients at risk for fluid overload unresponsive to medical therapy and preparation of red cell aliquots for infants who need small volume transfusions.

Washing red cells with one to two liters of 0.9 percent sodium chloride using an automated blood processor can remove up to 99 percent of the original plasma proteins. Manual washing by repeat addition of saline to the component and centrifugation is less effective in removing plasma but can be beneficial for patients with plasma protein reactions. Previously frozen red cells, after thawing and washing to remove the glycerol cryoprotectant, can be temporarily stored at 1 to 6°C but must be used within 24 hours since they are prepared in an open system and the risk for bacterial contamination exists. The washing of red cells or platelets to remove plasma also reduces the white cell content by approximately 90 percent. This method of leukoreduction is insufficient for the reliable prevention of febrile reactions human leukocyte antigen (HLA) alloimmunization and transmission of cytomegalovirus (CMV). Prevention of these conditions requires a 99.9 percent or more leukoreduction and can be accomplished by filtration using a leukoreduction filter. This has best results when performed near the time of donation prior to storage (prestorage leukoreduction), but can be performed in the hospital blood bank or at the bedside at the time of transfusion.

**Release of Blood Components from the Blood Bank Laboratory**

The issue or release of blood components from the blood bank inventory is an important multistep process to ensure that the appropriate unit is properly labeled and released for the correct patient. Immediately prior to release the blood component, previously determined to be crossmatch compatible with the patient if the component is red cells, is inspected by blood bank personnel to ensure that labeling is accurate and that the required testing and processing, such as irradiation, antigen typing, compatibility testing, etc., have been performed. In addition, a visual examination of the component is performed to confirm that there are no color changes, unusual cloudiness or large clots present to suggest bacterial contamination.

Blood bank records should document the date and time of release, the visual inspection and the persons involved. Hospitals should have a written procedure requiring that the person obtaining the blood component from the blood bank provide to the blood bank a form of identification of the intended recipient to assure the correct blood is released for the proper patient. This procedure also includes an audible
reading of patient and unit identifying information at the time of release. Blood components should be transported directly to the patient care area. Generally, if the component cannot be transfused promptly, it should not be released from the blood bank laboratory. If red cells are released and temporarily stored at room temperature but not used within 30 minutes, they should be returned to the blood bank for proper refrigerated storage.

**Blood Administration Steps**

The steps taken in administering a blood transfusion are outlined in Table 10.1. The first step is to verify the physician’s written order to be certain that the correct component will be transfused at the prescribed rate and that any special requirements, such as irradiation, are indicated by the proper label on the component.

**Patient Education**

Prior to the transfusion an assessment should be made as to the patient’s understanding of the procedure. Information and instruction should be given, verbally and in writing, if possible and if time permits. The procedure should be described so the patient knows what to expect. The patient should be encouraged to ask questions. The recipient should be informed what will take place and unless incompetent or unconscious, the patient should consent to the procedure. The patient should be asked if a previous transfusion has been received and if the patient has had any transfusion reactions. The symptoms of a reaction such as chills, shortness of breath, urticaria, pruritus and pain should be explained and the patient asked to report any symptoms immediately should they occur.

**Venous Access**

Venous access should be established and patency assured prior to obtaining the component from the blood bank. Ideally, a slow infusion of 0.9 percent sodium chloride using a Y-tubing set is used to maintain patency until the blood component is available.

The size of the infusion needle used should be large enough to assure appropriate flow rates. The recommended minimum needle sizes for adults are 18 to 20-gauge but smaller ones are used when the veins are too small for larger needles. The recommended minimum needle sizes for infants and children are 23 to 25-gauge. Small needles result in slow flow rates and use of pressure infusers through very small needles such as 23 to 27-gauge should be avoided to prevent hemolysis from occurring. Slow flow rates of red cell transfusions can be improved by diluting with 0.9 percent sodium chloride.

**Prophylactic Medications Given Prior to the Transfusion**

Most patients do not need premedication, but those with a history of allergic reactions such as urticaria should be given an antihistamine. Antipyretics may be given to patients with previous febrile transfusion reactions. The risk of febrile reactions to platelets may also be decreased by transfusing leukocyte-reduced components. Some patients with heart or renal disease can tolerate the volume of the transfusion better and avoid intravascular volume overload if given a
diuretic prior to or during the transfusion. The routine use of antipyretic and antihistamine premedications should be discouraged unless the patient’s history indicates the need.

**Infusion Supplies and Sets**

Blood components must be transfused through sterile, single-use, disposable, pyrogen-free plastic transfusion sets containing a drip chamber to monitor flow and an in-line filter to remove large clots and cellular debris that may have accumulated during component storage. Standard blood administration sets have an in-line filter with 170 to 260 microns pore size and the tubing should be primed and the drip chamber filled to above the filter prior to attaching the blood component. Only 0.9 percent sodium chloride should be used for priming the tubing. Other solutions may be incompatible and cause hemolysis or clotting.

No medication or solutions other than 0.9 percent sodium chloride can be added to the blood component. Five percent dextrose can cause red cell clumping and hemolysis. Lactated Ringer’s and other solutions containing calcium can cause clotting of blood components since the citrate anticoagulants used in blood components prevent clotting by binding calcium. Total parenteral nutrition solutions are hypertonic and hemolyze red cells.

Blood components should not be given at the same time as intravenous medications that are associated with a high rate of immediate adverse effects such as intravenous immune globulin, amphotericin, vancomycin, campath (alemtuzumab), antithymocyte globulin, etc. If medications with high rates of reactions are infused during blood component administration, a reaction to that medication can be mistaken as a transfusion reaction and the transfusion unnecessarily delayed or discontinued.

Y-type transfusion sets are more expensive but are preferred because they provide ease in priming the tubing set and allow for adding 0.9 percent sodium chloride for component dilution and flushing the line between multiple component administrations. Single line or straight sets can be used for single transfusions and be primed directly with blood, but this method provides less control over the infusion. It is best to have 0.9 percent sodium chloride available for all transfusions, for keeping venous access open and for further therapeutic use in case the transfusion needs to be stopped due to a reaction.

Although standard filters and infusion sets are capable of being used for multiple transfusions, it is generally advisable that a new administration set be used if the previous one has been in use for over four hours. This practice helps to avoid possible bacterial proliferation and maintain adequate flow rates.

**Blood Warmers**

The routine use of blood warmers is not needed for patients receiving transfusions. Blood warmers may be used for red cell and plasma transfusions to prevent hypothermia in patients receiving massive transfusions, in children receiving blood at a rate of greater than 10 ml per kg per hour and for patients with clinically significant cold agglutinins. Platelets are not transfused through a blood warmer. The hospital should have a written procedure for transfusing blood through a blood warmer. Blood must not be warmed above 42°C since excessive warming can cause hemolysis. Blood warmers must have a visible thermometer and a warning system to detect malfunction. This is usually an audible alarm that sounds before reaching a temperature at which red cells hemolyze. All blood warming devices must undergo routine, scheduled maintenance and this must include verifying the temperature alarm mechanism is working properly. Records of maintenance procedures, whether routine and for specific problems, should be available for review. Rapid warming can also be achieved by the addition of 0.9 percent sodium chloride that has been warmed to 70°C and added to an equal volume of red cells previously stored at 1 to 6°C without risk of hemolysis or adverse effect on red cell survival if transfused immediately after preparation. Adding heated saline is difficult to standardize or control; and if this method is used, it requires strict adherence to written standard procedures and should be carried out in the presence of the transfusing physician or blood bank medical director.

**Immediate Pre-Infusion Identification Steps**

Accurate identification of the patient and the intended blood component for transfusion may be the single most important step in preventing incompatible transfusions and severe and fatal reactions. Performing
these steps accurately and completely is imperative for safe transfusion but studies of the completeness of the final bedside check show room for improvement. A recent study of over 4,000 transfusions revealed a failure to match the patient’s wristband identification with the blood compatibility label in 25 percent of transfusions. Of acute hemolytic transfusion errors, 80 percent are due to clerical error such as misidentifying patients and the majority occur at the patient bedside. Hospitals must have procedures describing the blood administration process with clear steps for performing blood component and patient identification and correlation. Many hospitals perform ongoing audits of this process as a part of hospital safety and quality programs and as a part of hospital and blood bank accreditation requirements.

The person administering the blood transfusion along with one additional person, generally a nurse, together perform the final and critically important correlation of patient, blood component and transfusion record identification as the final barrier to erroneous transfusion. One person audibly reads the identifying information and both persons correlate the information on the patient wristband, transfusion record and blood component, including compatibility and expiration date information. Two very important identification steps are performed. First, the patient’s name and identification number on the patient wristband is matched to the patient name and number on the component compatibility record attached to the component. This establishes a link between the blood bank laboratory’s identification of the intended recipient (on the compatibility record attached to the component) and the actual recipient. Second, the component unique identification number on the component label is matched with the component unique identification number on the blood bank’s compatibility record attached to the component. This connects the laboratory’s identification of the specific unit of blood with the actual unit of blood itself.

In addition, the blood component and its label are examined at the bedside immediately prior to administering the component. These steps are very important not only to prevent hemolytic reactions to ABO incompatible blood but also to prevent other errors such as giving non-irradiated blood when irradiated blood was ordered by the physician for immunocompromised patients or giving the wrong component or one that had been stored beyond the expiration date, etc. (Table 10.2). The blood component should be examined for abnormal color, cloudiness and clots. The blood bank should be notified if there are any discrepancies or component abnormalities.

In some circumstances, a patient may not have visible, physical identifier such as a wristband. In the operating room, the armband may be covered by surgical drapes. Some hospitals perform transfusions on outpatients without armbands and this practice is strongly discouraged. Hospitals and physicians should have written procedures to assure accurate identification and matching of the patient and blood component in these clinical situations. To reduce the risk of bedside errors, methods are being developed for computer-assisted bedside identification of the patient’s bar-coded identification band and the bar-coded blood component.

### Table 10.2: Importance of final bedside examination of the component immediately prior to transfusion

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Correct identification of the patient, lab compatibility record attached to blood and blood component label by two persons</td>
</tr>
<tr>
<td></td>
<td>- Prevents wrong blood given to wrong person</td>
</tr>
<tr>
<td></td>
<td>- Prevents fatal hemolytic transfusion reaction due to ABO incompatibility</td>
</tr>
<tr>
<td>2.</td>
<td>Examine blood component label</td>
</tr>
<tr>
<td></td>
<td>- Expiration date: Prevents use of outdated blood</td>
</tr>
<tr>
<td></td>
<td>- Irradiation label with visual indicator change present showing that component was irradiated: Prevents use of non-irradiated blood when irradiated blood was ordered for an immune deficient patient</td>
</tr>
<tr>
<td></td>
<td>- ABO label: Prevents wrong ABO type from being infused.</td>
</tr>
<tr>
<td>3.</td>
<td>Examine the blood component</td>
</tr>
<tr>
<td></td>
<td>- Prevents use of blood with clots, hemolyzed blood, blood with abnormal color indication, bacterial contamination</td>
</tr>
</tbody>
</table>

### Infusion Steps

The patient’s temperature, blood pressure, pulse and respiratory rate must be obtained and recorded immediately (no more than 10 to 20 minutes earlier) before starting and at the end of the transfusion. The person infusing the blood should remain with the patient for at least the first 15 minutes of the transfusion to observe for adequate flow rates and the development of reactions. Unless urgently needed, the transfusion should be started slowly, e.g. 2 ml per
minute, then increased to the prescribed rate after being assured that there was no immediate adverse reaction developing. Patients with heart disease should receive transfusions more slowly, sometimes with use of a diuretic, to minimize the risk of volume overload and heart failure. It is generally advised that the temperature, blood pressure, pulse rate and respiratory rate again be obtained and documented after the first 10 to 15 minutes of the transfusion and at least hourly if the transfusion continues for more than an hour. To avoid risk of bacterial proliferation in the component, the duration of the transfusion should not exceed four hours.

Post-Infusion Steps

After completion of the transfusion, the time and date, volume infused, type of component, and the identity of the person stopping the transfusion should be recorded in the patient’s medical record. The patient should be monitored for signs and symptoms of a reaction for one hour after each transfusion. If the transfusion occurs as an outpatient and the patient departs for home after the transfusion is completed, a person who accompanies the patient should be instructed to be aware of the signs of a reaction and to observe the patient for at least one hour after the end of the transfusion. Some hospitals provide written instructions about the signs and symptoms of a reaction and what steps to take should a reaction develop. If there is no adverse reaction, the empty blood component container and tubing can be discarded in the appropriate biohazard trash containers. Some hospitals have a practice of storing the empty containers and tubing for a few hours in case they are needed to evaluate a transfusion reaction that develops shortly after the transfusion.

RECOGNITION AND MANAGEMENT OF REACTIONS

The nursing and medical staff performing the transfusion must be aware of the signs and symptoms of a reaction (Table 10.3) and be prepared to stop the transfusion, provide appropriate care and notify the blood bank laboratory. Signs of a serious reaction can be nonspecific and steps for managing these reactions should be performed consistently by all persons administering blood components. Some signs and symptoms such as fever, severe chills, respiratory distress, hypotension, and tachycardia may be similar regardless of whether the reaction is a hemolytic reaction or due to bacterial contamination or transfusion-related acute lung injury. Chills, low-grade fever and hypotension or hypertension can accompany the respiratory distress and pulmonary edema due to volume overload and heart failure. Even the hypotension, respiratory distress and respiratory failure due to an acute pulmonary embolism occurring during a transfusion can have symptoms similar to a transfusion reaction. Whenever these signs or symptoms develop, the transfusion should be stopped, the patient’s physician and the blood bank notified and the transfusion should not be restarted.

The hospital should have written procedures defining the specific signs and symptoms warranting cessation of the transfusion. Hospitals commonly set a minimum rise in temperature of 1 to 2°C or 2°F as a threshold that requires stopping the transfusion and initiating a suspected transfusion reaction evaluation. To prevent unneeded cessation of transfusions, it is recommended that a threshold for the resultant temperature also be set for triggering a transfusion reaction evaluation. For example, when a temperature rise of 1°C, 2°C or 2°F involves a pretransfusion temper-
rature of 35°C rising to a post-transfusion temperature of 37°C, it is not needed to stop the transfusion and initiate a blood bank evaluation of a suspected transfusion reaction. Some hospitals have required a temperature rise of 2°F or 2°C or more with a resultant temperature of 100°F or 101°F or 38°C or more before the transfusion is stopped. Setting specific temperature requirements that are practical is important so that transfusions are not stopped unnecessarily.

Whether a transfusion can be restarted after a febrile reaction is controversial with some arguing that after the blood bank shows that immune hemolysis is not the cause, the transfusion can be restarted if the patient’s physician concludes that the fever was caused by the patient’s underlying disease process. The practice of permitting the patient’s physician to decide to restart a transfusion has important drawbacks and can have serious consequences for the patient. Transfusion reaction symptoms are not specific; for example, the symptoms of a febrile non-hemolytic reaction can be the same as those caused by bacterial contamination of the blood component and continued transfusion of an infected component can be fatal. A negative Gram stain performed by the lab has limited sensitivity to detect bacterial contamination and has no ability to detect a high load of endotoxin that can be present. Endotoxin in bacterially contaminated blood components can contribute to a febrile reaction and cause fatal multiorgan failure. Febrile reactions can also be caused by leukocyte antibodies as part of transfusion-related acute lung injury. Restarting the same blood component could worsen the patient’s condition. The practice of not permitting the restarting of transfusions that were stopped due to febrile reactions is safer for the patient.

Some have argued that forbidding the restarting of transfusions will cause too much wastage of blood components. With the introduction of using leukoreduced components, it has been shown the rate of febrile reactions is low and stopping transfusions without restarting them does not contribute greatly to wastage. In a prospective study of 365 consecutive, leuko-reduced platelet transfusions to marrow transplant recipients, a febrile reaction rate of 1.4 percent was observed and when two transfusions where the temperature rose only from 97°F to 99°F were excluded, fevers to 100.1 and 101.7°F developed only in 2 of 365 or 0.7 percent (Unpublished observations. Eastlund: Fairview-University Medical Center, Minneapolis, 2003).

The only transfusion that can be restarted after a transfusion reaction is one during which the patient develops urticaria and pruritis and these signs and symptoms have been successfully treated with antihistamines. When urticaria and pruritus are accompanied by any other sign or symptom such as respiratory distress, bronchospasm, angioedema or hypotension, the transfusion should not be restarted even if the symptoms respond to therapy. In these cases, the blood bank should be notified and the patient and blood component investigated for cause. In most patients, a cause is not found and subsequent transfusions can be accomplished by giving antihistamines prior to the transfusions. In some patients, special components are necessary, e.g. IgA-deficient components, or components washed free of plasma proteins.

If not present during a transfusion, a physician should be immediately available in person or by telephone during the entire transfusion process. It is advisable that equipment for resuscitation and emergency medications be readily available during transfusions.

**Auditing Blood Component Transfusions**

Problems with blood administration and transfusion reactions should be regularly reported to and discussed by the hospital Transfusion Committee. In addition, periodic audits of blood administration are advised to identify areas needing improvement. Some hospitals audit a certain number of transfusions each month by having the component accompanied by blood bank personnel or another auditor using a standardized list of items to be monitored. A blood administration audit is often a standard part of blood bank and hospital accreditation procedures. Whenever the bedside identification check is observed to be performed incorrectly and observed during an audit, retraining of staff can occur and future transfusions are likely to be safer. Audit results, error rates and error analysis should be reported to the hospital Transfusion Committee.

In summary, the steps performed during the administration of a blood transfusion are critically important to providing a transfusion that is as safe as possible. Blood transfusions carry inherent risks
including immediate transfusion reactions and personnel carrying out transfusions must be prepared for the recognition and initial management of transfusion reactions.

REFERENCES

These practice guidelines have been developed by the medical staff at the Fairview-University Medical Center of the University of Minnesota Medical Center, Minneapolis, Minnesota, and are intended as an educational guide describing clinical circumstances in which blood components may be administered without further justification. The document includes conditions for which use of selected components is usually considered reasonable, but not mandatory, practice. They are not intended to serve as absolute medical indications. Not all patients eligible for blood transfusion by the guidelines will actually benefit from them. Likewise, transfusion therapy may be indicated for some patients who do not meet these criteria. An overly rigid adherence to these recommended indications could result in some patients receiving unneeded transfusions and others being under-transfused. Consultation with a transfusion medicine physician is recommended.

The clinician should be aware of the infectious and noninfectious risks, the alternatives and potential benefits of transfusions and only prescribe transfusions when the benefits outweigh the risks. The clinician should be prepared for the recognition and management of the acute adverse side effects and transmitted infections from transfusions. The clinician should record the reason for each transfusion and obtain informed consent prior to non-urgent transfusions.

### RED BLOOD CELLS

**Volume:** Approximately 300 ml.

**Dose:** In non-urgent settings, transfuse one unit at a time. For infants, 5 to 10 ml/kg can be given.

**Expected result:** One unit will increase the hemoglobin approximately 1 g/dl and the hematocrit by 3 percent in a 70 kg adult.

The major indication for red blood cell (RBC) transfusion is symptomatic anemia. Red blood cell transfusion restores the oxygen-carrying capacity of blood and thus alleviates the symptoms of tissue hypoxia. A patient’s hemoglobin level should not be the sole deciding factor in starting red cell transfusions. The decision to transfuse should be supported by the need to relieve clinical signs and symptoms and to prevent morbidity and mortality.

Do not transfuse red cells for volume expansion only or to enhance wound healing. A patient with a pharmacologically treatable anemia (i.e. iron, B₁₂, folate or other specific deficiency) should not be transfused, regardless of hemoglobin level, unless there are other risk factors for tissue hypoxia or symptoms severe enough to require immediate treatment. Except in exceptional circumstances, a patient with a hemoglobin > 10 g/dl is unlikely to benefit from a red cell transfusion.

The indications for transfusion of preoperatively donated autologous RBCs are equivalent to those for allogeneic blood.
RBC TRANSFUSION GUIDELINES — ADULTS

1. **Acute bleeding** ≥15 percent blood volume sufficient to produce signs of hypovolemia unresponsive to crystalloid or colloid infusions regardless of hemoglobin level. Note: Blood volume (ml) = wt (kg) × 70 ml/kg.

When deciding whether to transfuse RBCs to a patient who is actively bleeding, the clinical assessment of the rate or volume of blood loss (and, in particular, the development of signs and symptoms of hypovolemia) is more important than the hemoglobin level or hematocrit, since they may not reflect the degree of blood loss for many hours or until the patient is normovolemic.

2. **Symptomatic chronic anemia** (severe fatigue, weakness, shortness of breath, dizziness, chest pain, arrhythmia), if no other therapy is likely to correct the anemia.

3. **Asymptomatic chronic anemia**, if the patient is at increased risk of tissue hypoxia.

   Clinically significant tissue hypoxia does not usually begin to develop until the blood hemoglobin concentration is less than 8 g/dl, and transfusion above this level should be justified either by symptoms or by an underlying disease creating increased risk of tissue hypoxia. Such a disease might include, but is not limited to, symptomatic coronary artery disease, obstructive or restrictive pulmonary disease, or cerebrovascular insufficiency.

4. **Anemia with hemoglobin <8 g/dl** and impending surgery with expected blood loss.

5. **Hemoglobin <8 g/dl** with chronic anemia.

6. **Sickle cell disease** in selected clinical situations. Red cells are leukoreduced, < 14 days old and matched for Rh(cDeE), Kell(K1).
   a. Asymptomatic sickle cell disease if scheduled to undergo general anesthesia (may transfuse to 10 g/dl, but a hemoglobin of >10 g/dl or a hematocrit of >30 percent should be avoided due to potential complications of increased viscosity).
   b. Significant fall in hemoglobin from usual level.
   c. Exchange transfusion to reduce hemoglobin S levels to <30 percent (pregnant, CVA/TIA, seizure, chronic priapism, chronic non-healing ulcers, acute chest syndrome, non-resolution of pain crisis in selected situations).

RBC TRANSFUSION GUIDELINES — INFANTS AND CHILDREN

1. **Hemoglobinopathy**
   a. Patients with sickle cell disease who are symptomatic (cerebral symptoms; splenic, pulmonary or hepatic sequestration; priapism; or other sickle crisis) or require hypertransfusion/exchange transfusion.
   b. Asymptomatic sickle cell disease if scheduled to undergo general anesthesia or significant fall in hemoglobin from usual level.

2. **Acute blood loss**
   a. Loss of greater than 15 percent total blood volume in children >4 months of age (estimated blood volume = 70 ml/kg body wt) or greater than 10 percent blood volume in infants <4 months of age (est. blood volume = 85 ml/kg).
   b. With signs or symptoms of hypovolemia (tachycardia, tachypnea, hypotension) unresponsive to crystalloid or colloid infusion.
   c. Of lesser severity with signs and symptoms of cerebral or myocardial hypoxia, fatigue, weakness, dizziness or respiratory distress; other findings such as cardiomegaly, increasing liver size, rales, poor feeding, or apnea.

3. **Anemia**
   a. Symptomatic anemia regardless of etiology, if no other therapy (iron, folate, etc.) is likely to correct the anemia.
   b. Evidence of inadequate oxygen delivery.
   c. Hemoglobin <13 g/dl for term neonates <24 hours of age, premature neonates, patients with cardiopulmonary disease (in infants with symptomatic cyanotic heart disease, the hemoglobin level may need to be >13 g/dl), or infant <4 months of age prior to emergency surgery (in full term asymptomatic infants <4 months of age undergoing surgery, the hemoglobin level can be considerably less than 13 g/dl).
   d. Asymptomatic, hemoglobin <8 g/dl if deemed at high risk for development of symptomatic anemia under the following conditions:
      i. If no other medical therapy (iron, folate, etc.) is likely to correct the anemia.
ii. Emergency surgery scheduled in patients >4 months of age when time precludes evaluation of cause of anemia and specific replacement therapy.

iii. Surgical procedure in which significant blood loss is anticipated.

iv. Transfusion to maintain adequate oxygen delivery in patients with cardiopulmonary disease.

v. Therapy-induced myelosuppression.

4. *Exchange transfusion* for treatment of documented sepsis, hyperkalemia, hyperbilirubinemia, hemolytic disease of the newborn, drug overdose, liver failure, diffuse intravascular coagulopathy (DIC) or other coagulopathy, or congenital metabolic disorders.

FROZEN DEGLYCEROLIZED RED CELLS — ADULTS AND CHILDREN

1. Patients with multiple red cell antibodies needing rare blood types.
2. Autologous red cells for patients with antibodies to high frequency antigens.
3. Autologous red cells collected >42 days prior to intended use.

WASHED RED CELLS — ADULTS AND CHILDREN

1. Patients with immunoglobulin A (IgA) deficiency and antibodies to IgA.
2. Patients with haptoglobin deficiency and antibodies to haptoglobin.
3. Patients with repeated severe transfusion reactions to plasma proteins unresponsive to medications.

PLATELETS

*Volume:* Random donor platelet concentrates (from whole blood donation) 45 to 65 ml, apheresis platelets (from plateletpheresis donation using a cell separator) approximately 250 ml.

*Dose:* A pool of 5 to 6 concentrates is therapeutically equivalent to a plateletpheresis. The standard dose for adults is a pool of 5 platelet concentrates or a single plateletpheresis unit that will be freely substituted by the blood bank and issued for transfusion for inventory management purposes or when a pool of platelet concentrates is not available. For small adults and children, the dose is 1 platelet concentrate unit per 10 kg body weight. For neonates and infants, a dose of 5 to 10 ml per kg body weight is common.

*Expected result:* Transfusing a pool of 5 concentrates or a plateletpheresis should increase the platelet count by 25,000 to 50,000 μl in an average adult, in the absence of consumption.

*Note:* For patients not showing adequate response to platelet transfusion, or for indications for human leukocyte antigen (HLA) matched or crossmatched platelets, refer to Refractory Patient Guidelines.

Platelet transfusions are administered to stop or prevent bleeding associated with deficiencies in platelet number or function. Bleeding is much more likely to occur if thrombocytopenia is due to inadequate production (iatrogenic or disease-related) than immune destruction [e.g. idiopathic thrombocytopenic purpura (ITP)]. Patients with thrombotic thrombocytopenic purpura (TTP) or ITP should not be transfused unless there is bleeding. *Do not transfuse platelets* empirically with massive blood transfusion, or routinely following cardiopulmonary bypass, unless platelet count <100,000 μl.

PLATELET TRANSFUSION GUIDELINES — ADULTS AND CHILDREN

1. Platelet count <10,000/μl for the clinically stable patient with an intact vascular system and normal platelet function, prophylactic platelet transfusion may be indicated for platelet counts of <10,000/μl.
2. Platelet count <40,000/μl in patients with inborn errors of metabolism (e.g. Hurler’s syndrome) for 3 months following marrow transplant.
3. Platelet count <50,000/μl
   a. Severe active bleeding.
   b. Major surgery (preoperative and 48 hours post-operative) or impending invasive procedure. *A patient undergoing a surgical or other invasive procedure is unlikely to benefit from prophylactic platelet transfusion if the platelet count is 50,000/μl or more and thrombocytopenia is the sole abnormality.*
   c. Coagulopathy, including DIC.
   d. Newborn <1 month of age, or preterm infant.
   e. Patients with severe infections.
   f. Patients with sickle cell anemia while hospitalized following marrow transplant.
4. Platelet count <100,000/μl
   a. Following cardiopulmonary bypass or use of intra-aortic balloon pump or ventricular assist devices.
   b. Patients receiving extracorporeal membrane oxygenation (ECMO).
   c. Neurologic or ophthalmologic surgery.
5. Excessive bleeding regardless of platelet count in patients with cardiopulmonary bypass, ECMO, neurologic or ophthalmologic surgery.
6. Acute blood loss requiring more than one blood volume replacement (adult) or 50 percent blood volume replacement (children) within 24 hours, with platelet count <100,000 μl.
7. Documented or anticipated platelet dysfunction, regardless of the platelet count, if major surgery is anticipated, or if clinically significant bleeding occurs.

GUIDELINES FOR PLATELET TRANSFUSIONS IN REFRACTORY PATIENTS (Fig. 11.1)

1. Evaluation of patients refractory to platelet transfusion
   a. Determine whether the post-transfusion increment following administration of random donor platelet concentrates is satisfactory, e.g. using 1 to 24-hour post-transfusion platelet count alone, or by using a 10 to 60-minute post-transfusion count and documenting a corrected count increment (CCI) <5,000. A CCI <5,000 one-hour post-transfusion suggests immune factors (i.e. alloantibody) as contributory to refractoriness.
   b. Make a clinical judgment whether non-immune factors (sepsis, fever, antibiotics, bleeding, splenomegaly, etc.) are the major factors causing reduced platelet survival and refractoriness.
   c. If immune factors are thought to be contributory, order crossmatch-negative platelets. This requires sending a blood sample (EDTA, purple tube) to the blood bank.
   d. When crossmatch-negative platelets are ordered, also order the following: Platelet Antibody Screen and HLA Antibody Screen.

2. Selection of platelet components for refractory patients
   a. If not already used, a trial of fresh (<48 hr old) and ABO identical platelets is recommended.
   b. Crossmatch-negative platelets (single-donor apheresis platelets crossmatched with patient plasma): For the refractory patient with some incompatibility demonstrated in crossmatching, a trial of crossmatch-negative platelets is warranted. If there is no incompatibility, a pool of random platelet concentrate can be used.
   c. If platelet incompatibility is observed, the patient will be tested for platelet-specific antibodies and for HLA-antibodies.
   d. HLA-matched platelets: If there is no response to crossmatch-negative platelets, a trial of HLA-matched platelets is warranted. It may take 36-
72 hours to meet the first request for HLA-matched platelets (requires HLA typing, A and B locus, of patient; selection and recruitment of HLA-matched donor; donation using a blood cell separator for 2 or more hours, donor infectious disease testing, etc.).

e. If there is no response to crossmatch negative or HLA-matched platelets and HLA or platelet antibodies are present, further donor selection efforts should be made based on laboratory results and clinical circumstances (platelet antigen-negative donor if platelet specific antibody found; enhanced HLA donor selection based on identity of antibody specificity in patient; discontinuance of drug if implicated in antibody formation; potential family member donor, etc.).

f. If no response, and antibody tests are negative, random donor platelet concentrates are recommended.

\[ CCI = \frac{(post-platelet\ count\ minus\ pre-platelet\ count) \times body\ surface\ area\ (m^2)}{\text{number\ platelets\ transfused}\ (\times 10^{11})} \]  
(e.g. avg. transfusion is $4 \times 10^{11}$ platelets).

**FROZEN PLASMA**

*Volume:* Approximately 250 to 300 ml.

*Dose:* 10 to 20 ml/kg body weight.

*Expected result:* In a 70 kg adult, each unit will increase the activity of plasma clotting factors by about 4 to 5 percent, and fibrinogen by about 10 mg/dl.

Frozen plasma is administered to increase the level of coagulation factors in patients with single or multiple coagulation factor abnormalities when specific therapy is unavailable. Lab tests should be used to monitor the patient with a suspected clotting disorder and repeated (e.g. INR, PTT) following plasma transfusion. If the PTT is less than 45 seconds or INR less than 1.5, plasma transfusion is rarely indicated. Do not transfuse plasma for volume expansion, prophylactically with cardiopulmonary bypass, or as a nutritional supplement.

**PLASMA TRANSFUSION GUIDELINES — ADULTS AND CHILDREN**

1. **Suspected or proven coagulopathy** with PTT >45 seconds or INR >1.5, or laboratory test results pending.

2. **Active bleeding or prophylactically prior to surgery** in a patient with a coagulation factor deficiency, when specific factor concentrates are unavailable.

   Frozen plasma is a source of coagulation factors for patients with Factor II, V, VII, X, or XI deficiency for whom specific concentrates are unavailable and for patients with multiple coagulation factor deficiencies such as those with severe liver disease, DIC or vitamin K deficiency.

3. **Emergency reversal of Warfarin therapy** in a patient who is bleeding or requires emergency surgery, when time does not permit reversal by stopping the drug and administering vitamin K. Vitamin K can have an effect in 4 hours and INR normalized in 24 hours. Repeat INR after transfusion.

4. **Massive blood transfusion:** Bleeding with transfusion of more than one blood volume if PTT >45 seconds or INR >1.5, if test results available.

5. **Plasma exchange for TTP or hemolytic-uremic syndrome (HUS).**
6. Newborns who are septic and a coagulopathy is suspected.
7. Antithrombin III deficiency (if specific concentrate unavailable), or Protein C, Protein S, or Heparin Cofactor II deficiency.
8. Priming ECMO circuit in small patients.

CRYOPRECIPITATE-REDUCED (CRYO-POOR) PLASMA

Plasma, cryoprecipitate reduced (approximately 300 ml per unit), commonly called cryo-poor plasma, is fresh frozen plasma (FFP) that has been depleted of cryoprecipitable proteins [Factor VIII, fibrinogen, von Willebrand’s factor (vWF), Factor XIII]. Its use is for patients with TTP requiring plasma exchange.

CRYOPRECIPITATED ANTIHEMOPHILIC FACTOR (CRYOPRECIPITATE)

Component: One unit contains 150 to 250 mg of fibrinogen, 40 to 70 percent vWF, 80 to 120 units Factor VIII and 20 to 30 percent Factor XIII present in original unit.

Volume: Approximately 5 to 20 ml per unit.

Dose: According to clinical situation, one unit of cryoprecipitate per 10 kg body weight increases the fibrinogen level in the recipient by approximately 40 to 50 mg/dl.

Expected result: One unit will increase Factor VIII activity by about 4 percent, and fibrinogen by about 7 to 10 mg/dl in a 70 kg adult.

Cryoprecipitated antihemophilic factor (AHF), also known as cryoprecipitate (cryo) is the cold precipitable protein fraction derived from frozen plasma thawed at 1 to 6°C, and stored frozen at –18°C or colder. Cryoprecipitates contain Factor VIII, fibrinogen, vWF and Factor XIII.

CRYOTRANSFUSION GUIDELINES — ADULTS AND CHILDREN

1. Hypofibrinogenemia: Consumptive coagulopathy with recent or active bleeding or prior to invasive procedure or massive transfusion and fibrinogen level <100 mg/dl. Fibrinogen levels above 100 mg/dl are generally considered adequate for hemostasis.

2. Patients with von Willebrand’s disease (vWD) who are bleeding, when bleeding is unresponsive to desmopressin (DDAVP), or prophylactically prior to surgery.

The use of DDAVP is preferred, especially for patients with mild-to-moderate vWD (but not in patients with vWD, Type IIb, in whom DDAVP may cause platelet aggregates and thrombocytopenia). Humate, a factor concentrate, rich in vWF and treated with a viral inactivation step, is preferred to cryo due to Humate’s decreased infectious risk.

3. Fibrin surgical adhesive: Used as a topical adhesive and to stop vascular leaks in cardiovascular, orthopedic, ear, nose and throat (ENT), dental or neurologic surgeries. Commercial fibrin sealants available from the pharmacy have been treated with a viral inactivation step and may be preferred.

4. To enhance platelet function in patients with uremic platelet dysfunction and hemodialysis with active bleeding or prophylactically prior to surgery, if unresponsive to DDAVP or dialysis.

5. Factor VIII deficiency (Hemophilia A) if Factor VIII concentrates are not available, and no inhibitor present (Table 11.1).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level required for surgical hemostasis</th>
<th>Factor half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>100 mg/dl</td>
<td>72-120 hours</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>10-40%</td>
<td>72 hours</td>
</tr>
<tr>
<td>Factor V</td>
<td>10-30%</td>
<td>12-36 hours</td>
</tr>
<tr>
<td>Factor VII</td>
<td>10-25%</td>
<td>4-7 hours</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>• Major surgery, Bleeding: 80-100% • Postoperative: 30-50% • Minor bleed: 30-50%</td>
<td>8-12 hours</td>
</tr>
<tr>
<td>Factor IX</td>
<td>• Major surgery, Bleeding: 50-80% • Postoperative: 40% • Minor bleed: 30-50%</td>
<td>18-24 hours</td>
</tr>
<tr>
<td>Factor X</td>
<td>10-40%</td>
<td>24-48 hours</td>
</tr>
<tr>
<td>Factor XI</td>
<td>15-50%</td>
<td>40-84 hours</td>
</tr>
<tr>
<td>Factor XII</td>
<td>0%</td>
<td>48-52 hours</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>5-50%</td>
<td>9-12 days</td>
</tr>
</tbody>
</table>

6. Factor XIII deficiency.
7. Prolonged cardiopulmonary bypass or ECMO with active bleeding.
8. Newborns at significant risk for intracranial hemorrhage and fibrinogen level <150 mg/dl.

**GRANULOCYTE CONCENTRATES**

*Volume:* 150 to 300 ml.

*Dose:* The dose is a single granulocyte transfusion (collected by leukapheresis) per day.

Granulocyte concentrates are obtained from volunteer donors following dexamethasone stimulation and contain 2 to 3 x 10^10 granulocytes. Higher dose (e.g. 6–10 x 10^10) granulocytes for patients with infections unlikely to respond to standard dose may be available on research protocols by contacting the blood bank attending physician or transfusion medicine fellow.

Granulocyte concentrates also contain a considerable number of lymphocytes, platelets (equivalent to a single platelepheresis transfusion) and red cells (hematocrit 5–12%). Red cell compatibility testing is performed by the blood bank prior to transfusion.

Granulocyte concentrates are available only through consultation with a blood bank physician.

**GRANULOCYTE CONCENTRATE TRANSFUSION GUIDELINES — ADULTS AND CHILDREN**

1. Life-threatening bacterial or fungal infection in patients with neutropenia as documented by all of the following:
   a. Absolute neutrophil count < 500/μl (<1000/μl in neonates).
   b. Evidence of progressive infection, e.g.
      1. Fever >38.5°C for greater than 48 hours.
      2. Positive internal site cultures.
      5. Documented disseminated fungal infection.
   c. Broad-spectrum antibiotics (BSAs) or pathogen specific therapy has been given without clinical response.

2. Life-threatening infection in patients with granulocyte dysfunction unresponsive to antibiotics.

A single granulocyte transfusion is given daily until granulopoiesis returns, the infection is controlled, the patient is moribund or a contraindication develops.

Clinical effectiveness is diminished and reactions may be severe if the patient is alloimmunized and has leukocyte (granulocyte or HLA) antibodies. A test for granulocyte and HLA antibodies should be ordered and tested when starting a series of daily granulocyte transfusions.

Granulocyte transfusions *should not be infused at the same time* as infusions of medications that have a high incidence of causing adverse reactions [amphotericin B, intravenous immune globulin, Campath, interleukin-2 (IL-2), (ATG)]. Granulocytes can be given without delay immediately after amphotericin B.

Fever, rigors and pulmonary symptoms attributed to the granulocyte transfusion must be reported to the blood bank.

Because of a rapid decline in granulocyte function during storage, granulocyte concentrates must be transfused as soon as possible following donation, ideally within 12 hours and no later than 24 hours.

Because of this some blood donor infectious disease tests [human immunodeficiency virus ribonucleic acid (HIV RNA) and hepatitis C virus ribonucleic acid (HCV RNA)] will not be completed by the time of infusion, whereas the other donor tests will be completed and found negative [HIV-1 and HIV-2 antibodies, HIV antigen, HCV antibody, human T-cell leukemia virus (HTLV) antibody, hepatitis B surface (HBs) antigen, hepatitis B core (HBc) antibody, ALT and syphilis antibodies]. The estimated risk of acquiring HIV infection from the granulocyte transfusion prior to receiving results of blood donor RNA testing is 1 per 660,000 (instead of 1 per million if tested for HIV RNA) and for HCV infection 1 per 100,000 to 300,000 (instead of 1 per million if tested for HCV RNA). In some cases, a repeat donor is used, recently found negative for infectious disease testing, but test results from the date of donation will not be available by the time of transfusion. Consult with the blood bank physician about this to ensure accurate disclosure of risks to the recipient prior to granulocyte transfusions.

**LEUKOCYTE-REDUCED BLOOD COMPONENTS**

At most sites, cellular components are leukoreduced by the blood supplier at the time of donation and
bedside filtration to remove leukocytes is not needed. Cellular components are leukoreduced to levels required by the American Association of Blood Bank (AABB) and the Food and Drug Administration (FDA) to reduce the risk of cytomegalovirus (CMV) transmission, decrease the occurrence of non-hemolytic febrile transfusion reactions, reduce leukocyte antibody development in recipients and reduce the rate of refractoriness to platelet transfusions in multiply transfused recipients.

Non-leukoreduced cellular components can also be leukoreduced by transfusing through a leukocyte reduction filter at the bedside.

**LEUKOCYTE-REDUCED BLOOD COMPONENT TRANSFUSION GUIDELINES — ADULTS AND CHILDREN**

Leukoreduced cellular components provide these specific advantages:

1. Reduces the rate of febrile non-hemolytic transfusion reactions.
2. Prevents CMV transmission in patients at risk, who are suspected or documented to be CMV-seronegative.
3. Prevents or delays leukocyte alloimmunization.
   a. May delay or prevent febrile non-hemolytic reactions due to leukocyte antibodies. This is especially important to recipients likely to receive repeated transfusions, e.g. chronic anemias, hematologic malignancies.
   b. May delay or prevent development of platelet transfusion refractoriness due to HLA antibodies in patients with hematologic malignancies or aplastic anemia. This is especially important to recipients likely to receive repeated platelet transfusions.
   c. May prevent HLA sensitization in bone marrow, peripheral blood stem cell, cord blood, and solid organ transplant candidates or recipients.

**COMPONENTS WITH REDUCED CMV RISK**

Leukocytes, particularly lymphocytes and monocytes, present in red cell and platelet components, may carry CMV. Components (red cells and platelets) with reduced risk for CMV are leukoreduced to a level of $<5 \times 10^6$ leukocytes per component; these are provided by prestorage filtration leukoreduction, or bedside filtration leukoreduction. Leukoreduced components are therapeutically equivalent to CMV-seronegative components in reducing the risk of transfusion-transmitted CMV disease. Leukoreduction is not needed for acellular blood components such as plasma, cryo, albumin, immune globulin and coagulation factor concentrates.

**PATIENTS AT PARTICULAR RISK FOR CMV**

1. Pregnant (if the patient is CMV-seronegative or unknown).
2. Infants with birth weight $<1500$ gm until 4 months of age.
3. Patients with severe combined immunodeficiency disease (SCID) and other primary congenital immunodeficiencies.
4. CMV-seronegative solid organ transplant recipients.
5. CMV-seronegative bone marrow, peripheral blood stem cell, and cord blood transplant candidates and transplant recipients, regardless of donor CMV status.
6. HIV-positive patients who are CMV-seronegative [particularly if CD4 $<400$ or if the patient has acquired immunodeficiency syndrome (AIDS)].

**IRRADIATED CELLULAR BLOOD COMPONENTS**

Irradiation of cellular blood components containing viable lymphocytes is performed to prevent transfusion-associated graft-versus-host disease (TA-GVHD) in patients with profound immunodeficiency or suppression. Transfused viable lymphocytes of donor origin can proliferate and cause harmful immune reactions to the tissues of immunodeficient recipients. Irradiation is not needed for acellular components such as plasma, cryo, albumin, immune globulin and coagulation factor concentrates. Leukoreduction is not sufficient to prevent TA-GVHD. Irradiated cellular blood components need to be ordered for severely immunosuppressed patients to prevent TA-GVHD.

**GUIDELINES FOR IRRADIATED CELLULAR BLOOD COMPONENTS — ADULTS AND CHILDREN**

1. Irradiation is mandatory for:
   a. Bone marrow (and other hematopoietic progenitor cell) transplant patients (to begin no
later than admission for stem cell transplant or prior to preconditioning regimen).
b. Patients with congenital immunodeficiency syndromes.
c. All directed donations from blood relatives.
d. Bone marrow transplant donors: Allogeneic red cell transfusions should be irradiated if they are needed by the donor at the time of marrow donation, to prevent GVHD in the marrow recipient due to transfused allogeneic blood donor lymphocytes contaminating the donated marrow.
e. HLA-matched and crossmatch-negative apheresis platelets.
f. Intrauterine transfusions.
g. Patients who have received purine analogs (fludarabine, cladribine, pentostatin) or Campath in the previous 12 months.

2. Irradiation may also be considered for:
a. Any patient at increased risk for TA-GVHD, e.g. patients with therapy-induced immunosuppression, aggressive chemotherapy, extensive radiation therapy.
b. Septic neonates receiving granulocyte transfusions (buffy coats or leukapheresis).

COMPATIBILITY TESTING PRIOR TO TRANSFUSION (Tables 11.2 and 11.3)

Antibodies to red cells can develop following pregnancy (1–4% of multiparous females) or transfusion (e.g. 1% after a single transfusion and up to 30% of multiply transfused patients with chronic anemia) or can occur naturally (e.g. anti-A and anti-B in group O patients). To avoid hemolysis of transfused red cells, the blood bank performs the following:

1. ABO, Rh typing: For red cells, the blood bank selects blood that is the same ABO and Rh (D) type as the patient. Platelets are selected to be ABO identical with the patient, and if shortages exist, to be ABO compatible with the patient. Plasma and cryo contain anti-A or B and are selected by ABO blood group to ensure compatibility.

2. Antibody detection testing: The blood bank tests all prospective recipients for pre-existing unexpected red cell antibodies [e.g. antibodies to non-ABO antigens that are less immunogenic: Rh (C, c, D, E, e), Kell, Duffy, Kidd, etc]. If the patient has been transfused or pregnant in the past 3 months, this sample must not be > 72 hours old. Otherwise, the sample can be drawn up to 14 days prior to the transfusion.

<table>
<thead>
<tr>
<th>Turnaround time</th>
<th>Type of RBCs available</th>
<th>Testing performed</th>
<th>Risk for hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes (emergency)</td>
<td>O Neg RBC</td>
<td>None</td>
<td>Low*; life-threatening hemolysis rare</td>
</tr>
<tr>
<td>15 minutes (emergency)</td>
<td>ABO, Rh compatible type. RBC antibody screen not done</td>
<td>ABO, Rh type. RBC antibody screen negative; immediately spin or computer crossmatch performed</td>
<td>Negligible</td>
</tr>
<tr>
<td>30–45 minutes</td>
<td>ABO, Rh compatible (majority of patients lack RBC antibodies)</td>
<td>ABO, Rh type. RBC antibody screen performed</td>
<td>Negligible</td>
</tr>
<tr>
<td>2–36 hours</td>
<td>ABO, Rh compatible (patients with red cell antibodies)</td>
<td>ABO, Rh type. RBC antibody screen positive (full crossmatch performed)</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

Table 11.2: Compatibility testing

<table>
<thead>
<tr>
<th>Patient (recipient)</th>
<th>Compatible components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood group</td>
<td>Plasma contains</td>
</tr>
<tr>
<td>O</td>
<td>Anti-A, Anti-B</td>
</tr>
<tr>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>—</td>
</tr>
</tbody>
</table>

* Red cell antibodies are found in approximately 0.5 percent of patients (0.04% of persons who have not been transfused or pregnant, 1% of those previously transfused or pregnant, 3% of multiparous females)
Warning: For elective surgery, it is important to provide this sample at least one day prior to need for blood. If an antibody is found it may take 2 to 36 hours to identify the antibody and provide antigen-negative units.

3. Crossmatch: For the majority of patients (who have been tested and demonstrate absence of red cell antibodies), blood of the recipient ABO and Rh (D) type can be safely selected for the patient without a traditional crossmatch (patient serum mixed with donor red cells). However, because the most common transfusion-related death is from an acute hemolytic transfusion reaction during an erroneously given ABO-incompatible transfusion, special procedures are set up to ensure that the correct ABO type is released to the patient.

- The donor is typed at the time of donation and the donated blood is retyped when acquired by the hospital blood bank.
- The patient is ABO typed in duplicate and the patient’s record is searched to ensure current typing is identical to previous typing.
- For patients who have no unexpected red cell antibodies, the blood bank enters the red cell unit identifier into the computer which allows it to be released ONLY if the ABO, Rh of the unit matches the recipient’s ABO and Rh (“computer crossmatch”) stored in blood bank computerized records.
- At the bedside or in the operating room (OR), the patient’s identity (ID) band and the blood component are checked by at least two people to ensure that the patient’s name and ID number are identical on both.

For patients who have a positive red cell antibody detection test, or have a history of a red cell antibody that is not currently detectable, a full antihuman globulin crossmatch using recipient serum and donor red cells is required. If the crossmatch is negative, the red cells are released.

Blood bank red cell compatibility testing summary:
- Patient ABO, Rh typing
- Patient RBC antibody detection test
- RBC units selected to match ABO and Rh (D) and to be negative for other red cell antigen if antibody found in patients.

4. Final compatibility testing:
   a. If RBC antibody is currently present or had been detected previously, a full crossmatch is performed.
   b. If no RBC antibody is present, ABO/Rh compatibility of the patient and donor is confirmed prior to release.

Warning: If blood for compatibility testing was collected on the day of surgery, it is important to check with the blood bank or patient’s computerized record for blood availability prior to beginning surgery, because some patients are found to have unexpected red cell antibodies that could delay availability.

SOURCE OF BLOOD FOR TRANSFUSIONS

1. Allogeneic community-volunteer blood: Blood is provided from voluntary blood donors and the following steps are used to assure that the risks to the recipient are as low as possible.
   - Use of voluntary, unpaid donors
   - Medical and social history of donor (excluding donors with risk factors that may make the donated blood unsafe).
   - Physical exam of donor (exam of arms for “needle tracks” etc).
   - Confidential unit exclusion procedure by donor
   - Donor callback number provided.
   - Blood Testing:
     - ABO, Rh, red cell antibodies
     - HIV-1/HIV-2 antibodies
     - HIV RNA
     - Hepatitis C virus antibodies
     - Hepatitis C RNA
     - Hepatitis B core antibodies
     - Hepatitis B surface antigen
     - HTLV-I/HTLV-II antibodies
     - Syphilis antibodies
     - West Nile virus RNA
   - Recipient adverse reaction and transfusion-transmitted infection reports evaluated to exclude donor from future donations, if appropriate.

2. Autologous blood: Autologous blood donation provides the patient a method for avoiding transfusion-transmitted viral infection (HIV, HBV, HCV). Patients who are likely to need blood (e.g.
surgery that routinely uses an average of one unit of blood: hip revision surgery, etc.) can donate up to one unit per week at the blood supplier facility. Patients must be healthy (no serious cardiac or TIA/CVA risks), free of signs of infection and not anemic [hematocrit (HCT) must be 33%].

Because the infectious risks of allogeneic voluntary donor blood are so small and the risk of donating autologous blood is real (3% of donors have mild vasovagal reactions, 0.1% have prolonged syncope, seizures, other severe reactions, one in 7,000 has significant needlestick nerve injury, one in 14,000 needs hospitalization due to a reaction). The patient’s physician must balance risks and benefits to ensure blood is needed for surgery prior to requesting autologous donation. Nationwide, > 50 percent of autologously donated blood is collected but not used.

Because the infectious risks of blood transfusions are now so small, the most common fatal transfusion complications are hemolytic reactions due to transfusing the wrong ABO type, bacterial contamination of blood, and pulmonary reactions due to the presence of blood donor WBC antibodies transfused. These are not avoided by use of autologous blood.

3. Directed donation: Families or friends of the patient may donate for the patient. These directed donations allow friends and family to support the patient emotionally. Studies have shown that blood donated by family members or donors selected by the patient are not safer than blood derived from volunteer anonymous community blood donors. There is a probability that a mother of a newborn can provide blood with fewer risks than from community blood donors, but blood from others provides no added medical benefit.

RECOGNITION AND MANAGEMENT OF TRANSFUSION REACTIONS

1. Fever (temperature rise of at least 2°F and resulting in 101°F or more), chills, rigors, dyspnea, back pain, nausea, vomiting, tachy-bradycardia, hypohypertension — due to possible hemolysis/bacterial contamination/leukocyte antibodies.
   a. Stop the transfusion. Maintain IV patency.
   b. Assess the patient (check temperature, blood pressure, pulse, respiratory rate) and treat as needed.
   c. Recheck identity of blood component and recipient to see if wrong unit of blood was given.
   d. Initiate suspected transfusion reaction evaluation by blood bank.
   e. Send post-transfusion blood sample and return unused blood or empty bags to blood bank.
   f. DO NOT RESTART TRANSFUSION!
2. Plasma protein hypersensitivity — isolated hives (most common), generalized urticaria, angioedema, hypotension, bronchospasm.
   a. Stop the transfusion.
   b. Assess the patient.
   c. Treat as needed. For hives/urticaria give antihistamine. If hives and itching are the only symptoms and resolve, the transfusion can be restarted. For severe anaphylactoid reactions, epinephrine and steroids are needed.
   d. Initiate suspected transfusion reaction evaluation by blood bank unless hives/urticaria are the only abnormality and were treated successfully.
   e. Return unused blood or empty blood bags to blood bank.

MANAGEMENT OF ACUTE HEMOLYTIC TRANSFUSION REACTIONS (HTR)

After the post-transfusion blood sample has been examined by the blood bank for hemolysis (red color) and a direct Coombs'/direct antiglobulin test (DAT), the blood bank physician and patient’s physician will be notified if positive, indicating an acute HTR.
Acute HTR is often clinically mild, but the fatality rate is over 10 percent and aggressive treatment and close follow-up is advised. Acute renal failure may develop due to DIC, complement activation or hypotension. Immediate treatment should hydrate, maintain good urine flow, and maintain blood pressure. Steroids may lessen the immune reaction and complement activation.

1. IV fluids, saline 100 ml/hr for 24 hr or until red urine clears.
3. Furosemide for 40 to 120 mg IV, if urine flow less than 30 ml/hr.
4. Mannitol IV if above is ineffective.
5. Test and monitor for DIC: INR, partial thromboplastin time (PTT), fibrinogen, D-dimer, complete blood count (CBC), platelets.
6. Observe for pulmonary symptoms/ARDS.
7. Observe for hypotension and treat.
8. Observe for renal failure. Test urinalysis, blood urea nitrogen (BUN), creatinine, electrolytes.
9. Consider hydrocortisone, 150 mg IV Q6H × 2.

REPORTING TRANSFUSION REACTIONS

1. Except for simple isolated hives, report all reactions to the blood bank using the “Suspected Transfusion Reaction” form. Hospital nursing procedures are designed to ensure that all reactions in which the temperature rises 2°F or more (resulting in a temperature of 101ºF or more) during or within an hour of the transfusion are reported.
2. The only reaction that does not require reporting to the blood bank is simple urticaria and itching treatable by antihistamine. This is relatively common and occurs more often with plasma transfusion than with RBC or platelets. Only temporary discontinuance of the blood administration is required and it can be resumed after successful treatment of the urticaria. However, if hives are accompanied by wheezing, dyspnea, facial and tongue swelling (angioedema), hypotension or other symptoms, discontinue the transfusion and notify the blood bank.

RECOGNIZING TRANSFUSION-TRANSMITTED DISEASE

A history of recent blood transfusion should be sought when newly diagnosing any of the following infections which can be transmitted by transfusion in the USA: HIV, hepatitis A, B or C, West Nile virus, malaria, babesiosis, parvovirus, CMV, HTLV-I/II, bacterial sepsis, syphilis, chagas’, ehrlichiosis. **Recommendation:** Suspect transfusion-transmitted infection if the patient has no other likely cause and was recently transfused (within 6 months of the transfusion for hepatitis B and C, HIV and within four weeks of transfusion for West Nile virus infection).

REPORTING TRANSFUSION-TRANSMITTED INFECTION

Except for bacterial sepsis, which can present as an acute severe febrile reaction, these infections do not become apparent until weeks or months following the transfusion. This requires that the physician investigate whether the patient with newly diagnosed:

1. HIV, hepatitis, etc. had received a transfusion in the previous 6 months.
2. West Nile virus infection and received a transfusion in the four weeks preceding the onset of symptoms and if no other cause is likely, report it to the blood bank so the blood bank physician can investigate whether it was transfusion related and if so, report it to the blood supplier for further blood donor evaluation.

**Requirement:** Report every case of suspected transfusion-transmitted infection to the blood bank (HIV, hepatitis, bacterial sepsis, etc.).

RECOGNIZING TRANSFUSION-ASSOCIATED GRAFT-VS-HOST DISEASE

Transfusion-associated graft-versus-host disease (TA-GVHD) includes the usual signs of GVHD (rash, diarrhea, hepatitis) along with marrow aplasia and pancytopenia, which develops 8 to 10 days after transfusion and usually is fatal within 3 to 4 weeks.

**Reporting TA-GVHD**

If severe pancytopenia and clinical GVHD (rash, diarrhea, hepatitis) developed after a transfusion and a relationship to transfusion is suspected (e.g. non-BMT patient), report it to the blood bank.

**Reporting Transfusion-Related Fatalities**

If a transfusion is suspected to have caused a death, this must be reported to the blood bank. The blood
bank physician will investigate this and, if related, must report it to the FDA and to the blood supplier under federally required time restrictions.

INFORMED CONSENT

It is important and required that potential recipients of non-emergent transfusions of blood components be fully informed by their physician about the risks, alternatives (autologous donation) and potential benefits of blood transfusions. The hospital has informational pamphlets available for patients, describing the general nature of, risks from and alternatives to allogeneic blood transfusion. Quantitative estimates of transfusion risks follow.

Consent (discussion of risk, benefit, alternatives and decision to proceed) is obtained by the physician, whereas the patient’s signature can be obtained by a hospital staff member or a physician. Documentation of this informed consent must be found in the patient’s chart. The patient should sign the hospital “Affirmation of Consent for Medical and Surgical Procedure.” The hospital staff person or physician who obtains the signature also signs as witness.

The major difference between consent for blood transfusions and consent for surgical procedures arises for patients who require multiple transfusions. One consent form is sufficient for a single hospitalization, single episode of care or single treatment course. The physician can interpret what represents a single treatment course. Patients who are frequently transfused can have a single consent for a “course of treatment” and do not need a new consent for up to 12 months.

RISKS OF BLOOD TRANSFUSIONS — USA

Table 11.4: Blood transfusion risks

<table>
<thead>
<tr>
<th>Infectious disease</th>
<th>Risk per unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C virus</td>
<td>1 in 1.2 million¹</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>1 in 150,000¹</td>
</tr>
<tr>
<td>Human T-lymphotropic virus</td>
<td>1 in 641,000³</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>1 in 1.4 million¹</td>
</tr>
<tr>
<td>Bacteria in platelet concentrate</td>
<td>1 in 217²</td>
</tr>
<tr>
<td>Bacteria in apheresis platelets</td>
<td>1 in 357¹</td>
</tr>
<tr>
<td>Fatal bacterial sepsis, RBC</td>
<td>1 in 500,000 to 1 million</td>
</tr>
<tr>
<td>West Nile virus</td>
<td>Seasonal</td>
</tr>
<tr>
<td>Other infections (syphilis, malaria, babesiosis, Chagas’)</td>
<td>&lt;1 in 1 million²</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-infectious complication</th>
<th>Risk per unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hemolysis</td>
<td>1 in 15,600 to 35,700⁴</td>
</tr>
<tr>
<td>Fatal acute hemolysis</td>
<td>1 in 630,000⁴</td>
</tr>
<tr>
<td>Delayed hemolysis</td>
<td>1 in 4,000 to 11,600⁴</td>
</tr>
<tr>
<td>Fatal delayed hemolysis</td>
<td>1 in 3.85 million⁴</td>
</tr>
<tr>
<td>Febrile, non-hemolytic reaction</td>
<td>1 in 50 to 100⁵</td>
</tr>
<tr>
<td>Acute lung injury</td>
<td>1 in 5,000 to 100,000⁶</td>
</tr>
<tr>
<td>Hives</td>
<td>1 in 30 to 100⁷</td>
</tr>
<tr>
<td>Severe anaphylaxis</td>
<td>1 in 18,000 to 170,000⁷</td>
</tr>
<tr>
<td>Circulatory overload</td>
<td>1 in 3,000 to 12,000⁸</td>
</tr>
<tr>
<td>Transfusion-associated GVH disease</td>
<td>Unknown⁹</td>
</tr>
</tbody>
</table>

REFERENCES

Transfusions in Critically Ill Patients

Abram H Burgher
Jeffrey G Chipman

The efficacy of red cell transfusion for anemia in critically ill patients has met with considerable scrutiny of late. Studies indicate that the minimum required oxygen delivery is very low in both healthy and critically ill patients, suggesting futility of red cell transfusion except in certain, particularly unstable, clinical contexts.\(^1-3\) Compelling basic science and clinical investigations have proposed detrimental effects of blood transfusion, including microcirculatory dysfunction and immunomodulation.\(^4,5\) Large, prospective, randomized clinical trials in a variety of patient settings have demonstrated that liberal use of red cells is in most cases not beneficial, and may even be harmful.\(^6,7\) Taken together, these data support the notion that transfusions in critically ill patients are not without risk and that appropriate hemoglobin (Hb) thresholds for transfusion are lower than many had previously anticipated.

This chapter discusses current hypotheses and conclusions regarding anemia and transfusion practice in critically ill patients. The first two sections introduce the pathogenesis of anemia in critically ill patients, which stems from inadequate bone marrow production and shortened red cell survival. Following sections discuss oxygen delivery requirements and appropriate transfusion triggers (both generally and in specific subpopulations). Finally, the last two sections are comprised of a few selected topics of special interest, such as age of stored red cells, transfusion triggers in pediatric critically ill patients, and current transfusion practices in intensive care units (ICUs) in Western Europe and the United States.

**PATHOGENESIS OF ANEMIA**

**Impairment of Red Cell Production**

The pathogenesis of anemia in critically ill patients is multifactorial but can be reasonably divided into two major classes of dysfunction: reduced red cell production and enhanced consumption. Incompetent red cell production occurs in critical illness and the anemia associated with it resembles that seen in chronic inflammatory disease.\(^8\) Anemia is typically associated with measurable derangements in erythropoietin (EPO) production, iron metabolism and bone marrow function. This section introduces red cell production defects seen in critical illness, while the next focuses on enhanced consumption.

**Erythropoietin Expression and Responsiveness**

Erythropoietin (EPO) is produced by cells in the renal cortex and liver in response to hypoxia or reduced red cell mass.\(^9,10\) EPO, in turn, stimulates maturation of erythroid progenitor cells in the bone marrow, thereby increasing red cell production and circulating volume of red cells, and augmenting oxygen delivery (as discussed below in further detail, oxygen delivery is dependent in part on Hb concentration). Critically ill patients demonstrate dampened production of EPO, in spite of anemia, especially in the presence of sepsis or acute renal failure.\(^11\) Hypoxia-inducible factor (HIF)-1 is the putative transcription factor most important for EPO production in response to hypoxia. Investigations suggest that the increased oxidative
stress observed in critical illness may inhibit the action of HIF-1, thereby preventing a compensatory rise in EPO.\textsuperscript{12} Recently, weekly administration of recombinant human erythropoietin (rHuEPO) to critically ill patients was shown to reduce red cell transfusion and increase Hb, though overall an effect on mortality was not demonstrated (see discussion in special topics section).\textsuperscript{13}

**Iron Metabolism**

Because Hb requires iron to carry oxygen, proper iron metabolism is important for red cell production and function. As in anemia of chronic disease (ACD), anemia in critical illness is often associated with a functional iron deficiency (Table 12.1).\textsuperscript{9} Laboratory studies usually display low serum iron concentration, low transferrin saturation and elevated serum ferritin concentration. Studies in patients recovering from major surgery or those with multi-organ failure syndrome (MOFS) reveal similar findings.\textsuperscript{14,15} Alterations in iron metabolism appear to result from elevated inflammatory cytokines [especially tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1\(\alpha\) (IL-1\(\alpha\), and IL-6), which promote iron storage by stimulating production of ferritin, the principle iron storage protein.\textsuperscript{16} Additionally, regulatory cytokines elevated during critical illness (especially IL-4, IL-10, and IL-13) induce iron uptake by macrophages, further reducing serum iron.\textsuperscript{17,18} Finally, \textit{in vitro} studies indicate that EPO may be important in iron utilization.\textsuperscript{19} Since EPO is consistently low in critical illness, it may play an important role in the iron derangements seen in ICU patients.

**Nutritional Deficiencies**

Nutritional deficiencies occur in the ICU. Investigators have demonstrated deficiencies of iron (9\% of patients), vitamin B\textsubscript{12} (2\%), and folic acid (2\%) in critically ill patients.\textsuperscript{20} The clinical significance of these deficits is currently unclear, but each nutrient is known to be essential for the production of red cells.

**Enhanced Red Cell Consumption**

Like impaired red cell production, enhanced (or accelerated) consumption plays an important role in the development of anemia in ICU patients. Enhanced consumption, occurring by several means, reduces functional red cell volume.\textsuperscript{5} Oxidative damage to cell membranes during critical illness diminishes red cell lifespan. Occult blood loss, substantial phlebotomy, destruction of red cells due to contact with a mechanical device, and hypersplenism are all commonly encountered in ICU patients and each increases consumption or reduces functional volume of red cells (Table 12.2). As well, structural changes in red cells occurring during critical illness, such as reduced deformability, may limit oxygen delivery at the microcirculatory level and, while not directly contributing to anemia, perhaps result in a functional red cell deficit.\textsuperscript{5,9}

**Red Cell Aging**

Native red cells survive approximately 120 days in the circulation. They are removed from the circulation by splenic macrophages at the time of senescence. Among the natural consequences of red cell senescence are:

**Table 12.1:** Laboratory investigations reflecting deranged iron metabolism in critical illness

<table>
<thead>
<tr>
<th></th>
<th>Iron</th>
<th>Ferritin</th>
<th>Transferrin</th>
<th>EPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum value</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

**Table 12.2:** Etiology of accelerated consumption of native or transfused red cells in critical illness

<table>
<thead>
<tr>
<th>Method</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytosis</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td></td>
<td>Exposure of surface antigens</td>
</tr>
<tr>
<td></td>
<td>Reduced Hb content</td>
</tr>
<tr>
<td></td>
<td>Autoantibodies</td>
</tr>
<tr>
<td>Poor deformability</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td></td>
<td>Reduced ATP</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Increased intracellular calcium</td>
</tr>
<tr>
<td></td>
<td>Oxidative stress</td>
</tr>
<tr>
<td></td>
<td>Increased Intracellular Calcium</td>
</tr>
<tr>
<td>Poor oxygen delivery</td>
<td>Oxidative Stress (? MetHb)</td>
</tr>
<tr>
<td></td>
<td>Increased adhesion to endothelium</td>
</tr>
<tr>
<td></td>
<td>Increased 2,3-DPG</td>
</tr>
<tr>
<td>Destruction</td>
<td>Intra-aortic balloon pump</td>
</tr>
<tr>
<td></td>
<td>Ventricular assist device</td>
</tr>
<tr>
<td></td>
<td>Extracorporeal circuit</td>
</tr>
<tr>
<td></td>
<td>Prosthetic heart valve</td>
</tr>
<tr>
<td>Elimination</td>
<td>Phlebotomy</td>
</tr>
<tr>
<td></td>
<td>Occult bleeding</td>
</tr>
<tr>
<td></td>
<td>Hypersplenism</td>
</tr>
</tbody>
</table>
exposure of antigens at the red cell surface, which are recognized by autoantibodies; reduced antioxidant ability; apoptosis; decreased deformability; and altered oxygen uptake and unloading. While a number of possibly unique changes occur to stored blood (discussed in the special topics section), stored red cell units also simply contain a higher proportion of relatively old cells and a lower proportion of young cells, or reticulocytes, than would be found in vivo. For this reason, transfused red cells would, therefore, be expected to have a shorter average lifespan. Indeed, during prolonged refrigerated storage, an increasing proportion of red cells loses its capacity to survive in the circulation after transfusion.

Critical illness is thought to promote red cell aging by enhancing the normal signals of senescence. Phagocytosis by macrophages is at least partly dependent on the coating of red cells by auto-antibodies (specific for epitopes associated with senescence) and subsequent activation of complement. The accumulation of oxidized lipids and proteins on the red cell membrane may promote antibody binding and subsequent uptake by macrophages. Likewise, inflammatory cytokines, such as TNF-α, have been shown to reduce red cell lifespan, probably through enhanced macrophage uptake. The inflammatory state that often accompanies critical illness is associated with significant oxidative stress and elevations in a number of cytokines, including TNF-α. Therefore, it is reasonable to assume that phagocytosis of red cells may be upregulated.

Apoptosis

Red cells undergo apoptosis, or programmed cell death, in response to environmental signals, including oxidative stress. Recently, oxidative stress was shown to activate calcium-permeable channels in the red cell membrane, leading to an influx of calcium and subsequent apoptosis. Critical illness is associated with high oxidative demands on all body tissues and may, therefore, be associated with increased apoptosis of red cells.

Deformability

Since capillaries are typically smaller than red cells, proper flow requires deformation of an otherwise discoid red cell. Deformation within the capillary is thought to be dependent on a flexible cytoskeleton and membrane, both of which may be damaged in critical illness. Many states commonly seen in the ICU, including hemorrhagic shock, infection and sepsis, burns, cardiogenic shock, and ischemia-reperfusion injury, are associated with impaired red cell deformability. Laboratory studies on red cells collected from septic, critically ill patients reveal several lesions that may contribute to poor deformability in vivo: oxidative damage to the membrane; altered intracellular calcium concentrations; and decreased adenosine triphosphate (ATP). Reduced deformability may contribute to poor microcirculatory function and, as a consequence, exacerbate MOFS, but probably does not reduce survival of red cells.

Red Cell Destruction

Enhanced destruction of red cells is seen in a number of conditions, none specific to critical illness, but all commonly encountered in the ICU. Patients with congenital hemolytic disorders, such as sickle cell disease, red cell enzyme deficiencies, and hemoglobinopathies, often require red cell transfusion. Iatrogenic hemolysis is seen with intra-aortic balloon pumps, ventricular assist devices, prosthetic heart valves, cardiopulmonary bypass, and rapid infusion of hypotonic solutions. Additionally, hypersplenism, often encountered in conjunction with portal hypertension or splenic vein thrombosis but also seen in leukemia, can reduce intravascular red cell volume.

Blood Loss

Patients in the ICU are often subjected to a significant number of blood tests. Phlebotomy in North American ICUs averages more than one liter per patient during the ICU stay and is significantly correlated with transfusion. Phlebotomy is also higher in sicker patients and may account for up to 20 percent of total blood loss. A recent large, retrospective study demonstrated a positive correlation between organ dysfunction and number of blood draws. Clearly, only blood tests which are absolutely necessary and with minimum volume removed should be performed on the critically ill. Furthermore, when available, blood drawing devices aimed at minimizing discarded blood can and should be attached to central venous and arterial lines.
OXYGEN DELIVERY REQUIREMENTS

Systemic delivery of oxygen in critically ill patients can often be estimated (Table 12.3). It is primarily dependent on cardiac output, arterial oxygen tension and Hb content. However, oxygen delivery to peripheral tissues, which may be more meaningful, is difficult to determine with readily available clinical data. Effective peripheral oxygen delivery requires not only distribution to the capillary beds but also efficient transfer of oxygen from blood to cells and then to mitochondria within tissues. Though this chapter focuses primarily on increasing systemic oxygen delivery through the transfusion of red cells, it should be noted that an increase in systemic oxygen delivery does not necessarily result in a parallel increase of transport to peripheral tissues.

Oxygen Demand

The oxygen requirement of a given tissue depends on the organ and state of metabolism. Some organs—for example, the central nervous system—have routinely high oxygen demands, while others—skeletal muscle—have low requirements at rest but elevated needs during periods of exertion. Mitochondrial oxidation requires low oxygen tension—as low as 1 mm Hg within the tissues—but, since many cells are located at a significant distance from capillaries, if blood flow or oxygen content is limited, intracellular oxygen tension may fall below this value. If this happens, anaerobic glycolysis is favored over aerobic, leading to accumulation of lactate and other acids (as discussed below, while lactate may be elevated during periods of inadequate oxygen delivery, it is neither sensitive nor specific for ischemia in critically ill patients). Additionally, since anaerobic glycolysis produces much less energy (ATP) than aerobic metabolism, cellular energy supplies are compromised.

Critical Oxygen Delivery

“Critical oxygen delivery” is that which is minimally required to support the full demands of aerobic metabolism. Tissues receiving less than critical oxygen delivery rely to a greater extent on anaerobic metabolism and are said to be hypoxic. Detecting hypoxia in humans is often problematical; sensitivity depends on the measurement tool. There are global indices of oxygen deficit, like oxygen consumption and venous oxygen saturation, which are thought to be relatively insensitive since blood flow from tissue beds receiving sufficient oxygen delivery “dilutes-out” flow from hypoxic beds. More sensitive, but frequently not available in the clinical setting, regional measures of hypoxia include gastric tonometry (measuring intracellular pH), infrared spectrometry, oxygen electrodes, and magnetic resonance (MR) spectroscopy.

Based primarily on global indices, it appears that in health the level of critical oxygen delivery is very low and easily met. Critically ill patients, however, often have compromised cardiovascular and pulmonary systems, are many times anemic, and may have impediments to delivery of oxygen at the level of peripheral tissues. They may have relatively high oxygen demands because of ongoing inflammatory responses. It has been proposed, therefore, that the critically ill may be precariously close to critical oxygen delivery.

The effects of acute isovolemic Hb reduction have been evaluated in healthy, resting humans. In one study, the Hb of volunteers was decreased from average 13.2 g/dl to 5.0 g/dl, with a corresponding fall in oxygen delivery from 13.5 to 10.7 ml O2·kg⁻¹·min⁻¹. The investigators observed a predictable compensatory increase in cardiac output, but no increase in serum lactate, and no fall in oxygen consumption (Table 12.4) or findings of ischemia on electrocardiogram (EKG) tracings, even at the lowest Hb concentrations evaluated. In a subsequent study, oxygen delivery was reduced even further in healthy, resting humans with the use of acute isovolemic hemodilution and administration of a β-adrenergic antagonist, to 7.3 ml O2·kg⁻¹·min⁻¹, without ill effects. In healthy, resting humans, it would seem that critical oxygen delivery occurs at Hb<5 g/dl and below oxygen delivery of 7.3 ml O2·kg⁻¹·min⁻¹.

Table 12.3: Formula for determining systemic oxygen delivery

<table>
<thead>
<tr>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( O_2 \text{ content} = \left( \text{Hb g/dL} \times O_2 \text{ saturation} \right) \times (1.39) + 0.0031 \times (pO_2 \text{ mm Hg}) )</td>
<td>( O_2 \text{ content} ) = (Hb g/dL) \times O2 saturation \times (1.39) + 0.0031 \times (pO2 mm Hg)</td>
</tr>
<tr>
<td>( O_2 \text{ delivery} = \left( \text{Cardiac output} \right) \times (O_2 \text{ content}) )</td>
<td>( O2 \text{ delivery} ) = (Cardiac output) \times (O2 content)</td>
</tr>
</tbody>
</table>

Table 12.4: Formula for determining oxygen consumption

<table>
<thead>
<tr>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( O_2 \text{ consumption} = \left( \text{Cardiac output} \right) \times \left( \frac{O_2 \text{ content}<em>{\text{Arterial}} - O_2 \text{ content}</em>{\text{Venous}}}{O_2 \text{ content}_{\text{Arterial}}} \right) )</td>
<td>( O2 \text{ consumption} ) = (Cardiac output) \times \left( \frac{O2 \text{ content}<em>{\text{Arterial}} - O2 \text{ content}</em>{\text{Venous}}}{O2 \text{ content}_{\text{Arterial}}} \right)</td>
</tr>
</tbody>
</table>
One study examined critical oxygen delivery in septic and non-septic critically ill patients who were withdrawn from life support. The investigators monitored oxygen delivery and consumption as oxygen delivery was progressively reduced with sequential withdrawal of blood pressure support, inspired oxygen support and finally mechanical ventilation. Surprisingly, critical oxygen delivery was not reached until oxygen delivery fell below average 3.8 ml O₂·kg⁻¹·min⁻¹ in septic and 4.5 ml O₂·kg⁻¹·min⁻¹ in non-septic patients. However, patients enrolled in this study were all necessarily at a late stage of disease and many have argued that the results cannot be generalized to all critically ill patients, particularly those earlier in the course of their disease. It may be that critical oxygen delivery varies over the course of critical illness. A progressively decreasing critical oxygen delivery in critically ill patients may occur because oxygen consumption falls as organs and tissues cease functioning and blood is shunted to vital organs. Indeed, though many studies increasing oxygen delivery in the critically ill have failed to show benefit (Table 12.5), a strategy which augments oxygen delivery early in sepsis may be beneficial.

**APPROPRIATE TRANSFUSION**

Red cell transfusions come with benefits and risks, and each may be modified by clinical context. The putative benefit of red cell transfusion is an increase in oxygen delivery, with the intention of remaining above the critical oxygen delivery (described above) for all perfused tissues. Increasing oxygen delivery is the most commonly cited reason for transfusion.

Though there are a number of recognized risks associated with red cell transfusion—among them transmission of infection, which has been discussed elsewhere in this book—several exist which may be somewhat unique to critically ill patients: microcirculatory dysfunction, leading to organ failure; volume overload; and immunosuppression. Age of the patient, transfusion setting (perioperative versus medical intensive care unit), pre-existing disease, severity of current illness, and quality of the red cell unit transfused may all impact the balance of benefits and risks.

The Hb concentration below which benefits of red cell transfusion outweigh risks marks the ideal transfusion threshold and is often referred to as the “transfusion trigger”. Though there may be specific situations where even a small degree of anemia is poorly tolerated and a relatively liberal use of red cells is best, growing evidence suggests that in the majority of critically ill mortality and morbidity is best reduced with a restrictive transfusion policy (i.e. transfusion at relatively low threshold). This section will introduce data supporting restrictive transfusion policies, whereas the following section will discuss special situations, in which red cell transfusions may sometimes be used more liberally.

**Mortality**

Enough data exist from anemia in patients refusing blood transfusion to show that even very low Hb concentration is often tolerated, but that morbidity and mortality may be impacted by age, surgical procedure, or underlying disease, particularly cardiac. In 134 Jehovah’s witness patients (a religious group whose adherents usually refuse blood transfusion) with a Hb <8 g/dl or hematocrit (Hct) <24 percent who were treated for a variety of medical or surgical conditions, there were 23 reported deaths due to anemia. Of those deaths, 60 percent were in patients >50 years old. Yet, in 27 survivors with Hb <5 g/dl, 65 percent were <50 years old. Like age, type of surgical procedure may also impact morbidity and mortality seen with anemia. Patients undergoing high-risk vascular surgery are more likely to suffer a post-operative cardiac event with increasing anemia.

A study of 1950s Jehovah’s witness patients with cardiac disease demonstrated a >5-fold increase in...
mortality as preoperative Hb concentration fell from 10.9 g/dl to 6.0 g/dl.39

While retrospective studies have demonstrated an association between severity of critical illness, anemia and mortality, a multicenter, prospective, randomized trial in ICU patients did not show a survival benefit when red cells were transfused to keep Hb>10.0 g/dl. Investigators in this study, referred to as the Transfusion Requirements in Critical Care (TRICC) Trial, randomly assigned 838 ICU patients to receive red cell transfusions at a threshold of either 7.0 g/dl (restrictive policy) or 10.0 g/dl (liberal policy). A non-significant trend toward decreased 30-day mortality (18.7% versus 23.3%; p = 0.11) and a significant decrease in 30-day mortality among patients less critically ill (8.7% versus 16.1%; p = 0.03) was observed in the restrictive transfusion arm versus liberal. Similarly, in patients <55 years of age, a significant reduction in 30-day mortality (5.7% versus 13.0%; p = 0.02) was demonstrated in those randomized to the restrictive arm. A number of other randomized studies have compared liberal to restrictive transfusion strategies, but the TRICC Trial is the only one to date with sufficient statistical power to measure mortality in ICU patients. In aggregate, concerning both short-term mortality and total number of units transfused, prospective studies in a variety of clinical settings (with restrictive triggers ranging from 7.0 to 10.0 g/dl) demonstrate that a restrictive transfusion policy is as good as—if not superior to—liberal (Tables 12.6 and 12.7; TRICC Trial is in boldface).7

A large, retrospective study showed an association between red cell transfusion and mortality in patients with organ dysfunction. Higher mortality was demonstrated in transfused patients at all levels of organ dysfunction with the exception of those with the most severe organ failure.28 All other variables being equal, the investigators calculated that blood transfusion increased the risk of dying by a factor of 1.4.

### Table 12.6: Prospective studies randomizing patients to either liberal or restrictive transfusion strategy.
Relative risk refers to 30-day mortality in restrictive arm. Adapted from reference 7.

<table>
<thead>
<tr>
<th>Study</th>
<th>Setting</th>
<th>Restrictive (n/N)</th>
<th>Liberal (n/N)</th>
<th>Weight (%)</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blair 1986</td>
<td>GI bleed</td>
<td>0 / 26</td>
<td>2 / 24</td>
<td>0.7</td>
<td>0.19 (0.01, 3.67)</td>
</tr>
<tr>
<td>Bracey 1999</td>
<td>Cardiac surgery</td>
<td>3 / 215</td>
<td>6 / 222</td>
<td>3.1</td>
<td>0.52 (0.13, 2.04)</td>
</tr>
<tr>
<td>Bush 1997</td>
<td>Vascular surgery</td>
<td>4 / 50</td>
<td>4 / 49</td>
<td>3.3</td>
<td>0.98 (0.26, 3.70)</td>
</tr>
<tr>
<td>Carson 1998</td>
<td>Orthopedic</td>
<td>1 / 42</td>
<td>1 / 42</td>
<td>0.8</td>
<td>1.00 (0.06, 15.47)</td>
</tr>
<tr>
<td>Hebert 1995</td>
<td>ICU</td>
<td>8 / 33</td>
<td>9 / 36</td>
<td>8.6</td>
<td>0.97 (0.42, 2.22)</td>
</tr>
<tr>
<td><strong>Hebert 1999</strong></td>
<td>ICU</td>
<td>78 / 418</td>
<td>98 / 420</td>
<td>83.6</td>
<td>0.80 (0.61, 1.04)</td>
</tr>
<tr>
<td>Lotke 1999</td>
<td>Orthopedic</td>
<td>0 / 62</td>
<td>0 / 65</td>
<td>0.0</td>
<td>Not estimable</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td>100.0</td>
<td>0.80 (0.63, 1.02)</td>
</tr>
</tbody>
</table>

### Table 12.7: Prospective studies randomizing patients to either liberal or restrictive transfusion strategy.
Relative risk refers to units of blood transfused in restrictive arm. Adapted from reference 7.

<table>
<thead>
<tr>
<th>Study</th>
<th>Restrictive N</th>
<th>Liberal N</th>
<th>Weight (%)</th>
<th>Relative risk 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blair 1986</td>
<td>26</td>
<td>24</td>
<td>16.7</td>
<td>-2.00 (-3.30, -0.70)</td>
</tr>
<tr>
<td>Bracey 1999</td>
<td>212</td>
<td>210</td>
<td>22.0</td>
<td>-0.50 (-0.81, -0.10)</td>
</tr>
<tr>
<td>Bush 1997</td>
<td>50</td>
<td>49</td>
<td>16.7</td>
<td>-0.90 (-2.20, -0.40)</td>
</tr>
<tr>
<td><strong>Hebert 1999</strong></td>
<td>418</td>
<td>420</td>
<td>20.7</td>
<td>-3.00 (-3.64, -2.36)</td>
</tr>
<tr>
<td>Johnson 1992</td>
<td>20</td>
<td>18</td>
<td>21.0</td>
<td>-1.05 (-1.62, -0.48)</td>
</tr>
<tr>
<td>Topley 1956</td>
<td>12</td>
<td>10</td>
<td>2.9</td>
<td>-0.50 (-12.21, -0.79)</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>738</td>
<td>737</td>
<td>100.0</td>
<td>-1.63 (-2.67, -0.58)</td>
</tr>
</tbody>
</table>
Selected Outcomes

Although the weight of evidence suggests that a restrictive transfusion policy spares red blood cell (RBC) units and does not lead to an increase in short-term mortality (and may even be associated with a reduction), prospective, randomized trials have demonstrated varying impact of a restrictive policy on several measures of morbidity, such as myocardial infarction, infection, renal failure, and pulmonary edema (Table 12.8).\(^7\) One study reported that red cell transfusion may have a negative impact on pulmonary function in ventilated patients.\(^3^4\) In this study, mean duration of mechanical ventilation was no longer in patients assigned to a liberal transfusion strategy (8.7 days versus 8.3 days; \(p = 0.48\)), while a restrictive policy was associated with fewer pulmonary complications, specifically acute respiratory distress syndrome (ARDS) and pulmonary edema.

Special Situations

Cardiac Disease

Certain clinical situations may exist for which higher transfusion triggers are most appropriate. A retrospective study in the United States compiled data from the records of almost 80,000 patients older than 65 years with a primary diagnosis of acute myocardial infarction (AMI).\(^1\) Consistent with studies in patients with known cardiac disease who refuse blood transfusions, the authors found that lower admission Hct was associated with increased 30-day mortality. In fact, among patients with an admission Hct=27 percent, the 30-day mortality rate in those transfused was less than half that of those not transfused. Furthermore, with adjustment for clinical confounders, red cell transfusion was associated with a decrease in 30-day mortality among patients whose Hct on admission was less than 33 percent, but with an elevation in mortality at Hct>36 percent. It may be that an ideal Hb concentration range exists for patients with AMI.\(^3^4\) Since many with AMI will enter the ICU shortly after the cardiac event, a higher transfusion threshold than that used in the TRICC Trial should be considered in these patients.

Hypoxia

One prospective, randomized study of red cell transfusion in septic critically ill patients with hypoxia demonstrated the benefit of early goal-directed care based on central venous oxygen saturation.\(^4^2\) When Hct concentrations were increased to >30 percent in patients with venous oxygen saturation <70 percent, mortality fell from 46.5 percent in the control group to 30.5 percent in the treatment group. Not surprisingly, red cell use was significantly elevated in the treatment arm—64 percent of patients received a red cell transfusion, compared to 18.5 percent of controls—but the clear reduction in mortality should not go unnoticed. Critically ill patients with hypoxia may be candidates for elevated transfusion triggers, particularly early in the course of their disease.

Hypotension and Bleeding

In some clinical contexts red cell transfusion may be appropriate regardless of Hb concentration. In states of systemic inflammation and hypotension, associated with capillary leak of plasma into interstitial spaces; particularly, the lungs, red cells are sometimes transfused to maintain intravascular volume. Likewise, during acute bleeding episodes, especially those

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### Table 12.8: Prospective studies randomizing patients to either liberal or restrictive transfusion strategy.

<table>
<thead>
<tr>
<th>Study</th>
<th>Outcome measure</th>
<th>Restrictive ((n/N))</th>
<th>Liberal ((n/N))</th>
<th>Relative risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bracey 1999</td>
<td>Myocardial infarction</td>
<td>1/212</td>
<td>0/216</td>
<td>3.06</td>
<td>(0.13, 74.61)</td>
</tr>
<tr>
<td>Hebert 1999</td>
<td>Myocardial infarction</td>
<td>78/418</td>
<td>98/420</td>
<td>0.25</td>
<td>(0.07, 0.88)</td>
</tr>
<tr>
<td>Bracey 1999</td>
<td>Infection</td>
<td>5/212</td>
<td>3/216</td>
<td>1.70</td>
<td>(0.41, 7.02)</td>
</tr>
<tr>
<td>Bracey 1999</td>
<td>Renal failure</td>
<td>8/212</td>
<td>5/216</td>
<td>1.63</td>
<td>(0.54, 4.90)</td>
</tr>
<tr>
<td>Hebert 1999</td>
<td>Pulmonary edema</td>
<td>22/418</td>
<td>45/420</td>
<td>0.49</td>
<td>(0.30, 0.80)</td>
</tr>
</tbody>
</table>
Transfusions in Critically Ill Patients

153

involving the gastrointestinal tract (GIT), trauma, or surgical bleeding, red cells are often given in the face of pre-transfusion Hb that would otherwise be considered adequate. Clinical impression should dictate transfusion in these cases because Hb concentration, per se, may be less important or may not be available in a timely manner.

Lactic Acidosis

Despite evidence indicating futility, red cells are on occasion transfused in critically ill patients with lactic acidosis to correct presumed tissue hypoxia. However, elevation of lactate occurs by a variety of means, many of which cannot be corrected by increasing oxygen delivery with red cell transfusion. Etiologies of lactate elevation in the critically ill are:

1. Hypoxic causes, in which anaerobic production of lactate occurs globally (e.g. shock) or focally (e.g. bowel infarction), and
2. Non-hypoxic causes (e.g. delayed lactate clearance with hepatic dysfunction, accelerated aerobic metabolism, and dysfunction of pyruvate dehydrogenase due to thiamine deficiency or endotoxin). Lactate has proven an ineffective measure of tissue hypoxia and response to increase in oxygen delivery. As such, in the absence of clear evidence for an etiology of lactate elevation, its levels should not be used to guide red cell transfusion.

Acute Respiratory Distress Syndrome

Some investigations of patients with acute respiratory distress syndrome (ARDS), sepsis and congestive heart failure (CHF) have reported that critical oxygen delivery (described in the section on oxygen delivery requirements toward the beginning of this chapter) might occur at a relatively high value. Specifically, studies in ARDS initially indicated that red cell transfusion in the face of otherwise clinically sufficient oxygen delivery, especially in the presence of elevated lactate, might increase oxygen delivery and oxygen consumption, indicating enhanced uptake of oxygen by peripheral tissues and theoretical benefit of transfusion. However, subsequent investigations revealed that deficiencies in statistical analysis were responsible for the observed effect. Because both oxygen delivery and consumption had been calculated using shared variables, associations between the two were erroneously attributed. It is now agreed that patients with ARDS do not generally benefit from red cell transfusion in the absence of clinical hypoxia. In fact, as described above, red cell transfusions may contribute to pulmonary dysfunction.

SPECIAL TOPICS

Stored Red Cells

Changes occurring in stored red cell products, referred to as the “storage lesion”, are well documented and involve alterations not only to the red cells themselves but also the storage medium (Table 12.9). Corpuscular modifications include loss of cell membrane and oxidative damage, while changes to product supernatant include mainly accumulation of various bioactive substances. Red cells deprived of energy (ATP), as occurs during storage, undergo disc-sphere transformation:

Table 12.9: Changes occurring in stored red cells (storage lesion*) which may impact critically ill patients.

<table>
<thead>
<tr>
<th>Biochemical alteration</th>
<th>Proposed functional defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpuscle</td>
<td></td>
</tr>
<tr>
<td>↑ Proportion of spherocytes</td>
<td>Impaired deformability</td>
</tr>
<tr>
<td>Oxidation of membrane</td>
<td>Early senescence</td>
</tr>
<tr>
<td>Oxidation of cytoskeleton</td>
<td>Impaired deformability</td>
</tr>
<tr>
<td>↓ 2,3-DPG</td>
<td>Impaired oxygen unloading</td>
</tr>
<tr>
<td>↑ Methemoglobin</td>
<td>Impaired oxygen loading</td>
</tr>
<tr>
<td>Supernatant</td>
<td></td>
</tr>
<tr>
<td>↑ Cytokines</td>
<td>Febrile reactions/immunomodulation</td>
</tr>
<tr>
<td>↑ Histamines</td>
<td>Febrile reactions/immunomodulation</td>
</tr>
<tr>
<td>↑ Bioactive lipids</td>
<td>Pulmonary injury</td>
</tr>
</tbody>
</table>

* Prestorage leukoreduction may modify, but not eliminate, many of these alterations
the proportion of spherocytes rises in proportion to storage time.\textsuperscript{46} Oxidative damage to cytoskeletal proteins and membrane phospholipids also occurs.\textsuperscript{47} As a consequence, stored red cells are poorly deformable and exhibit increased osmotic fragility.\textsuperscript{46} Intracellular changes comprise conversion of Hb to met-Hb and depletion of 2,3-DPG in 7 to 14 days, hindering the loading and unloading of oxygen in transfused red cells, respectively.\textsuperscript{48} Many of the corpuscular changes are corrected within 24 hours \textit{in vivo} after transfusion.\textsuperscript{49,50} Nevertheless, collectively, these alterations may briefly limit transit of transfused red cells through the microcirculation and reduce oxygen delivery to peripheral tissues.\textsuperscript{5,51}

The storage medium may also exhibit changes in proportion to storage time. Bioactive substances, such as histamine, lipids, cytokines, and soluble human leukocyte class I antigens, have all been identified in the supernatant of red cell products.\textsuperscript{35} (Antileukocyte antibodies may also be found in the supernatant of blood products and are thought to contribute to transfusion-related acute lung injury; their levels or potency do not increase in parallel with storage time.\textsuperscript{55}) Increased levels of cytokines have been documented in a variety of blood products, even those leukoreduced prestorage.\textsuperscript{52,53} It has been known for quite some time that elevated cytokines in blood products are associated with febrile reactions during and after transfusion. However, recently, the accumulation of bioactive lipids and cytokines (particularly IL-6) has been implicated in transfusion-related acute lung injury, a much more dangerous complication.\textsuperscript{34} The transfusion of stored red cells may also be related to pulmonary dysfunction after cardiopulmonary bypass.\textsuperscript{55} Changes in the supernatant during storage may compromise microcirculatory flow and activate inflammatory cells, particularly neutrophils.\textsuperscript{35}

Critically ill patients, who may have diminished cardiorespiratory reserve, and often exhibit a systemic inflammatory response, may be particularly vulnerable to the red cell “storage lesion”.\textsuperscript{5,56} One retrospective study illustrated the potential harm of red cell transfusion in critically ill patients with sepsis—transfusion was not associated with an increase in oxygen transport to peripheral tissues but instead with microcirculatory dysfunction. The authors demonstrated an inverse relationship between age of transfused blood and change in gastric intramucosal pH (a surrogate measure of intestinal hypoxia), such that red cells greater than 15 days were consistently associated with post-transfusion gastric ischemia.\textsuperscript{5} Studies in rats have also shown a lack of increase in peripheral oxygen transport and uptake after transfusion of relatively old red cells (though it may be that red cell physiology in rats is significantly different from humans).\textsuperscript{51} These findings inspired the question: should only fresh red cells be used in critically ill patients?

Recently, a prospective trial sought to answer this question.\textsuperscript{56} Patients were randomized to receive red cells stored for \( \leq 5 \) or \( \geq 20 \) days. Among other measurements, gastric intramucosal pH, arterial pH and arterial lactate concentration were followed. No significant differences were observed among those receiving old cells and those receiving fresh. The authors proposed several explanations for differences between their findings and earlier studies, but among the most compelling are the use in the more recent study of only prestorage leukoreduced blood products and the enrolment of septic patients at a later stage in the course of disease. Therefore, although the red cell storage lesion is well documented, its clinical relevance remains unclear.

**Pediatric Critically Ill**

Major trials of red cell transfusion thresholds in critically ill patients have by and large enrolled adult patients only. A pediatric trial (TRIPICU) analogous to the TRICC Trial,\textsuperscript{6} which randomized patients to a liberal or restrictive transfusion strategy, is ongoing and its results are eagerly anticipated. Still, some data already exist to support relatively low transfusion thresholds in critically ill children. One investigation in pediatric ICUs and trends in the TRICC Trial lend credence to the emerging belief that a restrictive red cell transfusion strategy may be best in children as well as adults.

A retrospective investigation evaluated red cell transfusion in patients from five pediatric intensive care units in North America, enrolling those experiencing Hbs\( \leq 9 \) g/dl at some time during their ICU stay.\textsuperscript{58} Patients were divided into two groups:

1. Those receiving red cell transfusion immediately after falling below this Hb threshold, and
2. Those not receiving a transfusion. After controlling for the effects of other variables, transfusion was
associated with a statistically significant increase in days of mechanical ventilation, length of hospital stay and days of vasoactive infusion; and a non-significant trend toward higher mortality. Subsequent editorials in pediatric journals have suggested that a reflexive trigger of Hb≤9 g/dl is probably not ideal, and that the appropriate transfusion threshold in pediatric critically ill patients may be impacted by primary diagnosis, hypoxia, coagulopathy, and cyanotic heart disease, among others. A small, observational study of children at our institution did not indicate benefit of red cell transfusion on mortality or ventilator weaning with pretransfusion Hb≥12 g/dl (data not published). Certainly, results from the coming randomized study (TRIPICU) are much anticipated and should include subgroup analyses.

Often, critically ill children suffer volume overload and, occasionally, will require volume reduced blood products. Since platelet products contain only a very small volume of platelets (but a much larger volume of suspension medium), total volume can be significantly reduced via centrifugation and resuspension in a smaller volume. It should be noted, however, that this process may reduce radiolabeled recovery. Packed red blood cells cannot be volume reduced to any great extent, since the Hct of a packed red cell unit is about 80 percent. When transfusions of red cells are required in volume overloaded patients, diuretics or dialysis are usually necessary.

Blood Conservation

Blood products are usually available in limited quantity and even when available are expensive. Because critically ill patients frequently require significant blood product support, methods to minimize transfusion are especially important in the ICU. A variety of approaches exist to reduce transfusion requirements in critical illness: administration of nutritional support, since vitamin deficiencies are not uncommon in the critically ill; avoidance of bone marrow toxins; erythropoietin administration; therapy with red cell alternatives, like Hb solutions; and minimization of unnecessary blood tests, since phlebotomy is directly associated with likelihood of red cell transfusion and may, in fact, be the most significant independent predictor of subsequent transfusion.

Not surprisingly, arterial blood gases are the most commonly ordered blood test in North American ICUs and may account for up to 40 percent of total blood withdrawn during a patient’s ICU stay. Volume phlebotomized per draw is usually small—1.5 to 10 ml per laboratory test for basic chemistry and coagulation studies—but cumulative removal can be significant. The risk for phlebotomy-related anemia is greatest for critically ill pediatric and neonatal patients, whose total blood volume is small. As such, blood conservation methods are widely practiced in many pediatric ICUs. Some of these practices can be converted to use in an adult setting. In adult ICUs, the use of pediatric tubes for most routine blood tests—limiting the amount of blood that can be withdrawn for any given test—has been shown to decrease total phlebotomy by 42 percent.

In the postoperative period, some critically ill patients will have significant blood loss from surgical drains. This blood, maintained steriley, can sometimes be salvaged. However, because it is diluted, partially hemolyzed, and contains fibrin degradation products and high levels of cytokines, the utility of such salvaged blood is not clear. Blood can also be salvaged intraoperatively. It is typically mixed with heparin, concentrated by centrifugation, washed with saline, and reinfused. Generally, red cell salvage is contraindicated in surgery for cancer or that involving the aerodigestive or genitourinary tract for fear of reinfusion of tumor cells or bacteria. Still, in selected patients—those with very large postoperative blood loss and transfusion requirements—collection and autotransfusion of blood may limit the development of anemia and reduce allogeneic red cell transfusion.

Erythropoietin Administration

As detailed at the beginning of this chapter, the critically ill exhibit a blunted erythropoietin response to anemia. Because of this, investigators sought to determine if exogenous, recombinant human erythropoietin (rHuEPO) could ameliorate anemia in critically ill patients. It was found that weekly administration of rHuEPO could raise hemoglobin while reducing the likelihood of transfusion and total number of red cell units transfused, when compared to control. Interestingly, even though as a whole there was no significant difference in mortality among treated and control groups, patients in the treatment arm who
were less ill showed a significant reduction in mortality. In addition, the treatment group showed a non-significant trend toward reduction of respiratory insufficiency and MOFS, but an increase in cardiac events. (These data seem to support the hypotheses that red cell transfusion may be associated with some degree of pulmonary impairment in critically ill patients, and that the risk-benefit ratio favors a restrictive transfusion policy in young, less ill patients.) Weekly administration of rHuEPO, while expensive may, therefore, mitigate the need for red cell transfusion in ICU patients. Certain subgroups may especially benefit, whereas others may incur slightly greater risk.

CURRENT TRANSFUSION PRACTICES

As detailed throughout this chapter, a number of studies have provided evidence concerning appropriate transfusion practices in the critically ill. Some studies have even evaluated subgroups of patients; for example, those with cardiac disease, ARDS, or hypoxia in sepsis. In light of the mounting evidence for specific and relatively low transfusion thresholds, it would be expected that transfusion practices have changed to incorporate these new findings.

Two recent multicenter studies evaluated ICU transfusion practices in a large number of patients in a variety of ICUs. One of these, a retrospective investigation, evaluated red cell transfusion practices in critically ill patients in Western Europe. It was found that less than 30 percent of transfusions were given at Hb ≥ 9 g/dl, a practice seemingly consistent with recommendations derived from data contained in the only large, prospective randomized trial evaluating transfusion thresholds in the critically ill, the TRICC Trial. Interestingly, pretransfusion Hb was similar in a variety of conditions, indicating that physicians were likely transfusing on the basis of a prefigured threshold instead of adjusting for clinical context (Table 12.10). In the United States, a prospective investigation studied red cell transfusion practices in ICUs. In this study, mean pretransfusion Hb concentration was 8.6 g/dl and similar among all reported indications for transfusion, comparable to practices in European ICUs. The results of these two investigations evaluating transfusion practices support the notion that recent data demonstrating the benefit of relatively low transfusion thresholds for most critically ill patients have impacted clinical care.

Table 12.10: Stated indications for transfusion in patients in Western European ICUs. Adapted from reference 28.

<table>
<thead>
<tr>
<th>Indications for ICU transfusions</th>
<th>Transfusions No. (%)</th>
<th>Pretransfusion Hb Mean, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute bleeding</td>
<td>702 (55.5)</td>
<td>8.4</td>
</tr>
<tr>
<td>Inadequate Hb with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diminished physiologic reserves</td>
<td>355 (28.0)</td>
<td>8.4</td>
</tr>
<tr>
<td>Altered tissue perfusion</td>
<td>213 (16.8)</td>
<td>8.4</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>104 (8.2)</td>
<td>8.7</td>
</tr>
<tr>
<td>Other indications</td>
<td>142 (11.2)</td>
<td>8.4</td>
</tr>
</tbody>
</table>

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37. Viele MK, Weiskopf RB. What can we learn about the need for transfusion from patients who refuse blood? The experience with Jehovah’s witnesses. Transfusion 1994;34:396-401.


Blood Transfusion in the Operating Room

David Beebe  
Kumar Belani

Patients undergoing major surgery often require transfusion of blood components to maintain hemodynamic stability and allow blood clotting to occur. Although blood loss occurs from the surgery, the anesthesiologist is generally responsible for the administration of blood components in the operating room. Therefore, it is essential for both anesthesiologists and surgeons to have an understanding of when to transfuse patients and what steps to take to prevent major morbidity and mortality if massive hemorrhage occurs.

The first decision faced by physicians when bleeding occurs in the operating room is whether red cells need to be administered at all. Infectious diseases of donor origin such as HIV, hepatitis B and C viruses, and malaria, etc. remain to be a risk for transfusion recipients despite of our best efforts at screening blood donors. Both major (ABO incompatibility) and minor (urticaria, fever, chills) transfusion reactions may also occur. Transfusion-related lung injury due to leukocyte antibodies in the blood donor or the recipient can occur even when only small quantities of blood components are administered. In addition, several studies suggest that a blood transfusion is an immunosuppressive agent. While this may be beneficial for patients undergoing organ transplantation who need to be immunosuppressed, blood transfusions can result in an increased morbidity and mortality from tumors or infections in patients with an intact immune system. Therefore, the decision whether to transfuse a patient should always made deliberately with consideration of the risks and benefits.

GUIDELINES FOR TRANSFUSION

In the past, arbitrary guidelines were used for determination if blood should be administered during surgery. For example, blood was arbitrarily administered if the blood loss was ten percent or greater. Often, blood was administered with its attendant risk for complications in patients that did not need it. A more judicious approach can be used based upon an understanding of how patients respond to acute hemorrhage and a reduction in the hemoglobin level.

In general, most patients can tolerate an acute reduction of their hemoglobin concentration by blood loss to 10 gm/dl provided adequate volume with crystalloid or colloid is administered. Because Ringer’s lactate and other crystalloid solutions rapidly equilibrate with the extracellular fluid and the total amount of extracellular fluid is three to four times the blood volume, three to four times the measured blood loss of crystalloid solution must be administered. Colloids such as 5 percent albumin may also be used; but because it does not leak out of the intravascular space, it can be used to replace blood loss ml per ml. However, colloids are much more expensive than crystalloid solutions, can precipitate allergic reactions and are not always readily available. A reduction of a hemoglobin level to 10 gm/dl does not result in harm to most patients as long as the intravascular volume is adequately maintained with crystalloid or colloid solutions. The viscosity of the blood becomes lower as the hemoglobin falls and as more blood is pumped by the heart to maintain oxygen delivery to the tissues, the lower viscosity of the blood may contribute some to lessening the work for the heart. If the hemoglobin
level falls below 10 gm/dl; however, the work of the heart and its oxygen consumption will have to increase to maintain adequate circulation and oxygen delivery to the tissues. In young healthy patients this is no problem. Hemoglobin levels of 7 gm/dl are well tolerated. However, in older patients or those with coronary disease, myocardial ischemia may result.

One method that is useful to help determine when to administer red cells is to initially calculate the patient’s circulating blood volume. This varies by age and gender. In general, men have a higher blood volume by weight (approximately 70 ml/kg) than women (60 ml/kg). The amount of blood that will cause a 10 percent drop in hemoglobin (i.e. 10% of a blood volume) can then be calculated. The amount that can be lost until a predetermined hemoglobin is reached can thus be calculated. For example, a 70 kg man has an approximately five liter blood volume (70 ml/kg times 70 kg body weight is equal to 4,900 ml). If his starting hemoglobin is 15 gm/dl, a 10 percent loss (500 ml) can result in a 10 percent reduction in the hemoglobin concentration to 13.5 gm/dl. A further 10 percent loss of 500 ml would reduce the new hemoglobin concentration of 13.5 gm/dl by 10 percent or to 12 gm/dl, and so on. Thus, a healthy young individual as provided in this example can tolerate almost a 2 liter blood loss before his hemoglobin level drops to less than 10 gm/dl, and an additional liter loss will result in a hemoglobin of 8 gm/dl. The amount that can be lost in small women is obviously much less.

**Standard Precautions Taken in the Operating Room**

Care must be taken when administering red cells in the operating room that the donor and recipient have the same blood type. The blood type of the units transfused should be checked by at least two people prior to its administration. In the case of massive hemorrhage, where fully cross-matched blood is not available, type-specific blood may be administered. While this does not prevent hemolytic reactions due to non-ABO antibodies detectable in the cross match, these are relatively rare. O negative blood may also be used in emergencies because it does not have the A or B antigens on the red cells. However, the plasma of O negative blood can have significant hemolytic anti-A and anti-B antibodies that can result in hemolysis of recipient’s red cells containing A or B antigens. Therefore, packed red cells, not whole blood, should only be used in this situation to minimize exposure to potentially incompatible plasma. If more than two units of O negative blood has been transfused, the patients should not be transfused with his proper blood type until the blood bank has determined that the recipient anti-A and anti-B levels from the O-negative blood have fallen to safe levels.

**Recognition of ABO Incompatible Transfusion**

Unfortunately, despite these precautions, major transfusion reactions from administration of ABO incompatible blood in the operating room still occur. It is often difficult for the anesthesiologist to immediately recognize and treat a transfusion reaction in the operating room because an anesthetized patient will not be able to complain of chills, fever, chest pain or nausea. Under general anesthesia, the only signs are often hypotension, a bleeding diathesis and red urine due to hemoglobinuria. Disseminated intravascular coagulation precipitated by the lysis of red cells and renal failure from the effects of free hemoglobin, red cell stoma and cytokines released from hemolysis of the packed red cells may result.

If a transfusion reaction is suspected, the anesthesiologist first must stop the blood transfusion. The unused blood component should be sent to the blood bank for red cell compatibility and bacterial contamination. A sample of the patient’s blood should be sent to the blood bank for an antibody screen and direct antiglobulin (Coombs) test. In addition, blood should be sent to the laboratory for the plasma hemoglobin concentration, clotting studies and a platelet count. A urine sample should be tested to determine whether the red urine is from hemoglobinuria suggesting hemolysis or hematuria with intact red cells from another cause. Treatment of the reaction itself is primarily supportive, i.e. the blood pressure must be maintained to ensure adequate tissue perfusion and urine flow. To prevent renal failure, a brisk diuresis should be initiated with intravenous fluids, mannitol (12.5–25 grams) and a diuretic such as furosemide (20–40 mg). Alkalinizing the urine by administering 40 to 70 mmol of sodium bicarbonate may be beneficial to prevent precipitation of the acid hematin in the distal kidney tubules.
Problems Related to the Storage Lesion

Red cells are living cells. Blood that is stored to be used for blood transfusion in the operating room has certain characteristics and biochemical abnormalities that are important for the anesthesiologist to know, particularly if large amounts of blood are required. The pH of stored red cells progressively decreases from a value of 7.55 when it is collected to less than 7 by 30 days or so. The potassium progressively increases to a level of 20 to 30 mmol/L by one month. The level of 2,3-diphosphoglycerate declines progressively, transiently reducing the ability of the red cells to unload oxygen to the peripheral tissues.

To keep blood or plasma from clotting, sodium citrate is added to the blood. Sodium citrate binds calcium. Calcium is an essential element for functioning of the enzymes in the coagulation cascade and binding it stops the blood from clotting. Packed red cells are stored cold to preserve the life of the red cells. Also, since most patients who require blood still have adequate coagulation enzymes, fibrinogen and platelets well to provide normal homeostasis in spite of losing blood, packed red cells rather than whole blood are administered. Even during surgery, most normal patients who require a blood transfusion do not need the plasma or platelets found in a unit of fresh whole blood. Therefore, when blood is collected from donors, these components are removed and made into platelet and plasma components for use by other patients who need them.

Even though stored packed red cells are cold, acidotic, hyperkalemic, contain sodium citrate, and have a few platelets or clotting factors, they can be used for most patients undergoing blood transfusions. However, in the operating room, where blood components are administered rapidly and in large quantities, these factors can become important. Sodium citrate from banked blood that is administered rapidly (>200 ml/min) can reduce the ionized calcium level. A diminished ionized calcium level can cause myocardial depression and hypotension. Calcium chloride (5–10 mg/kg), sodium bicarbonate (1–2 mmol/kg), plus D5W (25 gm) and insulin (10–20 units) are required. In extreme cases, one may deliver inhaled beta-agonists via the endotracheal tube (albuterol inhaler solution). These efforts will decrease acute life-threatening hyperkalemia.

Blood should be warmed to body temperature if it is administered rapidly. Hypothermia resulting from the administration of cold blood components can result in worsened coagulation, postoperative shivering and rigors as well as myocardial depression and ischemia and possibly an increased incidence of wound infection. Warming of the blood components prior to administration in the operating room is particularly important if the blood is transfused using a central venous catheter. Cold blood administered directly in the heart can decrease the compliance of both ventricles of the heart acutely and result in myocardial depression.

Blood Product Replacement Protocol in the Operating Room

When a patient requires a transfusion, the anesthesiologist and surgeon must decide if blood components such as plasma or platelets are required in addition to the packed red cells. In general, most patients can have one blood volume replaced with packed red cells and fluid such as lactated Ringer’s solution or 5 percent albumin before dilutional coagulopathy develops and hemostasis becomes a problem. Prior to administering blood components such as plasma, platelets or cryoprecipitate, the wound edges should be examined to see if the patient is clinically coagulopathic. If there is no significant bleeding and the surgery is complete, plasma and platelet components may not be needed in spite of abnormal laboratory values.

If the patient appears coagulopathic and component therapy is being considered, several laboratory tests can be helpful if available. During trauma surgery with massive blood loss and transfusions, thrombocytopenia may develop from dilution by resuscitation fluid and packed red cells. If the platelet count is less than 100,000 per microliter and the patient is actively bleeding, platelets should be administered. Generally, one donor unit of platelets derived from one whole-blood donation will raise the platelet count by 10,000
per microliter in an adult. Usually, five to six platelet units are administered at a time for thrombocytopenic patients that are actively bleeding.

Surgical patients can become coagulopathic from dilution of clotting factors including fibrinogen following resuscitation with packed red blood cells, colloid or crystalloid solution. Generally, most patients have adequate reserves of these proteins so that the coagulation cascade is not significantly affected until at least one blood volume is transfused. However, if the patient appears coagulopathic and the platelet count is adequate, several laboratory tests may be of benefit. If the prothrombin time and INR is prolonged to greater than 1.5 to 2 times normal and the patient is actively bleeding, plasma should be administered. A prolonged prothrombin time indicates a deficiency of clotting factors most of which are produced by the liver. Patients with liver disease or who suffer from nutritional deficiencies may have a reduction in these enzymes and not have the reserve necessary to achieve adequate clotting following surgical blood loss.

A second important test that may be helpful in evaluating whether clotting factors must be administered during surgery where bleeding is taking place is the fibrinogen level. Fibrinogen is required to make fibrin, and a deficiency that occurs in liver disease or following massive hemorrhage can result in a persistent coagulopathy. Fibrinogen can be partially replaced when the patient is given fresh frozen plasma (FFP). However, in cases of severe deficiency and fibrinogen is less than 100 to 150 mg/dl and there are persistent losses from hemorrhage, cryoprecipitate may be required. Cryoprecipitate contains much more fibrinogen, factors VIII and von Willibrand’s factor (vWF) per unit volume than FFP so it is more effective in rapidly correcting fibrinogen deficiency.

Other tests may also be helpful in evaluating coagulopathies that develop in surgery. If heparin has been administered to the patient as part of the procedure, an elevated activated clotting time or partial thromboplastin time suggests the heparin has not been adequately reversed. Protamine administration, not blood components alone, is appropriate in this situation.

Some institutions utilize the thromboelastogram to diagnose the cause of intraoperative coagulopathies. The thromboelastogram is measured using a sample of the patient’s whole blood sitting in a small cup that oscillates back and forth. As the blood clots, it causes a pin suspended in the blood to move. The speed of clotting and strength of the clot is then determined and can be plotted graphically. The pattern of the graph can help clinician determine if the patient needs platelets, plasma or cryoprecipitate.

The thromboelastogram is also useful in diagnosing fibrinolysis. Fibrinolysis can result in coagulopathy during surgery because the clots are lysed by the fibrinolytic system prematurely. Fibrinolysis can occur from conditions causing disseminated intravascular coagulation such as a septic uterus or can be seen in severe liver disease. The treatment of disseminated intravascular coagulation and primary fibrinolysis during surgery is difficult. The cause of the disseminated intravascular coagulation must be eliminated or corrected, if possible. Drugs such as e-aminocaproic acid or aprotinin can be administered to inhibit fibrinolysis. The anesthesiologist must then work with the surgeon and blood bank physicians to get adequate blood components into the patient to achieve hemostasis.

In the future, new and exciting developments in transfusion therapy may help eliminate or reduce the need for blood transfusions in the future. Factor VIIa can now be made with recombinant DNA technology. Administration of this factor has stopped major hemorrhage in surgical cases where standard transfusion practices proved ineffective. Similar technology is likely to alter how and when anesthesiologists administer blood components in the future.

Transfusion of Blood Components during Surgery in Infants and Children

The proper care of premature newborn infants, young infants and children during surgery requires the use of blood components during surgery and the perioperative period. As in adults, the circulating blood volume in premature newborn infants, infants and children is based upon body weight; and thus even though blood loss may be small when measured in actual milliliters, but it may be a large portion of the circulating blood volume. Thus, pediatric anesthesia providers have to be ready to provide blood component therapy during surgical procedures resulting in significant blood loss during surgery in this age group. The circulating (estimated) blood volume of premature newborn infants, infants and children is
listed in Table 13.1. Table 13.2 lists the surgical procedures that most commonly result in significant bleeding in these age groups that require blood loss replacement.

**Table 13.1. Estimated blood volume (EBV) in premature newborn infants, infants and children**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Estimated blood volume (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature newborn infants</td>
<td>90 – 100</td>
</tr>
<tr>
<td>Newborn</td>
<td>80 – 90</td>
</tr>
<tr>
<td>Infant &lt; 1 yr</td>
<td>75 – 80</td>
</tr>
<tr>
<td>Child &gt; 1 yr</td>
<td>70 – 75</td>
</tr>
</tbody>
</table>

**Table 13.2: Surgical procedures that most commonly require blood transfusion**

- Craniofacial reconstruction
- Open heart surgery
- Posterior spinal fusion
- Sacrococygeal and other teratomas
- Cystic hygroma and other vascular tumors in the head and neck
- Liver and kidney transplantation
- Wilms’ tumor resection

Pediatric patients will maintain a near normal blood pressure in the face of as much as a 25 percent decrease in their circulating blood volume because of a very strong sympathetic drive that results in significant vasoconstriction. In premature newborn infants and infants, tachycardia is a prognostic indicator of shock. A decrease in blood pressure in such situations suggests impending cardiovascular collapse. Anesthesia providers must ensure normovolemia in premature newborn infants and infants to avoid such catastrophes. In premature newborn infants and infants, this is done by a close monitoring of bleeding and initially using crystalloid solutions for the first 5 to 10 percent blood loss in a 3 : 1 ratio. When blood loss approaches 10 percent, colloid solutions are started in a 1 : 1 ratio and a decision has then to be made about providing red cells for oxygen transport. Since infants and children do not have underlying cardiorespiratory and vasculopathies seen in adults and the elderly, red cells are used more stringently in this age group. The decision to transfuse red cells is often made by the clinician based upon a combination of factors namely hemodynamic status, stage of surgery and pre-existing cardiopulmonary disease and overall metabolic status of the infant and child. Thus, transfusions are started sooner in premature newborn infants than in infants and sooner in infants than in children. If bleeding is significant, thrombocytopenia may develop and a decision has to be made about adding platelet transfusions. Table 13.3 will help the anesthesia provider to decide about red cell and platelet transfusions.

**When does one use Plasma?**

This is a clinical decision that has to be made individually by practitioners and often is a collective decision between surgeon, anesthesia provider and sometimes the hematologist. When there is a known deficiency of clotting factors such as in infants and

**Table 13.3: Use of hemoglobin levels and platelet counts to guide use of red cells or platelets during surgery**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Premature newborn infants and infants</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use red cells for low</td>
<td>• &lt;13 g/dl (Hct &lt; 40%) with severe</td>
<td>• &lt;13 g/dl (Hct &lt; 40%) with severe cardiopulmonary disease</td>
</tr>
<tr>
<td>hemoglobin level</td>
<td>cardiac pulmonary disease</td>
<td>• &lt;8 g/dl (Hct &lt; 24%) during surgical bleeding</td>
</tr>
<tr>
<td></td>
<td>• &lt;10 g/dl (Hct &lt; 30%) with moderate</td>
<td>• Acute blood loss &gt; 25% EBV</td>
</tr>
<tr>
<td></td>
<td>cardiac pulmonary disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• &lt; 10 g/dl (Hct &lt; 30%) during</td>
<td></td>
</tr>
<tr>
<td></td>
<td>surgical bleeding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Acute blood loss &gt; 25% EBV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• &lt; 50 – 100 × 10⁹/L and significant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bleeding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• &lt; 50 × 10⁹/L following surgical</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bleeding and RBC + fluid replacement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Platelet dysfunction by thromboelastogram or other test despite adequate platelet count function</td>
<td></td>
</tr>
<tr>
<td>Use platelets for low</td>
<td>• &lt; 50 × 10⁹/L and significant</td>
<td></td>
</tr>
<tr>
<td>platelet counts</td>
<td>bleeding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• &lt; 50 × 10⁹/L following surgical</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bleeding and RBC + fluid replacement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Platelet dysfunction by thromboelastogram or other test despite adequate platelet count function</td>
<td></td>
</tr>
</tbody>
</table>
children with advanced liver disease, plasma is used throughout surgery for blood loss replacement. In these situations, it can be reconstituted with red cells to provide coagulation support along with oxygen-carrying capacity. This provides an adequate amount of circulating clotting factors. In infants and children without liver disease, when blood loss exceeds one blood volume, coagulation tests are abnormal and more surgical bleeding is anticipated, practitioners can add plasma to red cells and platelets for blood loss replacement. At the same time, platelet count, INR, prothrombin time with or without thromboelastography are done to monitor coagulation function. A frequent assessment of hemoglobin and hematocrit levels is done to guide red cell therapy.

Compared to adults, the transfusion risks of hypocalcemia, hyperkalemia and hypothermia during rapid replacement of surgical blood loss in premature newborn infants, infants and children are more likely to occur. Cardiac depression due to hypocalcemia from the binding of ionized calcium from citrate in the preservative solution can result in hypotension and bradycardia despite a normal or raised central venous pressure. Thus, monitoring of ionized calcium levels is important and hypocalcemia is directly related to the volume and rate of blood replacement. Large amounts of stored blood transfusion can also result in hyperkalemia. This can be minimized by active diuresis or sometimes by the use of washed red cells as used in the care of infants and children during liver transplantation. Hypothermia can be minimized by using blood warming systems (e.g. Level-1 blood warmers). Hypomagnesemia has also occurred following massive transfusion in this age group and thus magnesium levels must be monitored to correct this situation. Finally, transfusion-associated graft-versus-host disease (GVHD) although rare, can occur following transfusion of cellular blood components to immunodeficient patients but can be prevented by gamma irradiation of the component.

SUMMARY

Anesthesiologists are responsible for blood product administration in the operating room and should have a clear understanding of its risks and benefits in adults, premature newborn infants, infants and children. With this understanding, decisions in the operating room can be rapidly and properly made that can benefit patients.

BIBLIOGRAPHY

Patients who receive large volumes of red cells along with other blood components quickly present management challenges not seen in patients receiving just a few units of red cells in the same period of time. The distinction between those receiving large volumes and those receiving standard transfusions, however, is not precise. The three most commonly used definitions of massive transfusion (MT) are (i) replacement of 50 percent of the blood volume within 3 hours, (ii) replacement of a blood volume within 24 hours, and (iii) transfusions of more than 20 units of red cells. Since the management challenges seen with MT require constant monitoring of laboratory data and therapeutic decisions, the above definitions do not allow timely identification of MT episodes. The person most qualified to monitor these variables is the director of the transfusion service.\textsuperscript{1,2} For these reasons, a working definition of MT has been adopted so that the transfusion service director can be notified at the start of an MT episode. This definition is as follows: a patient who requires 4 units of red cells within an hour with anticipation of ongoing usage. The protocol we have developed to aid the transfusion service director manage MT episodes is included at the end of the chapter.

**PHYSIOLOGY OF BLOOD LOSS**

Knowledge of the clinical findings and physiology associated with hemorrhage is required to manage MT episodes. The American College of Surgeons Committee on Trauma has defined four clinical classes of patients based on the amount of blood they have lost (Class I–IV).\textsuperscript{3} Table 14.1, modified from their publication, describes the clinical findings for these classes.

When hemorrhage starts, the body begins to compensate by transferring extracellular fluid to the vascular space along with some vascular contraction. That is why, 15 percent blood volume loss (Class I) can be tolerated without change in the clinical status of the patient. With greater than 15 percent blood loss (Class II), cardiac output begins to fall resulting in decreased pulse pressure, tachycardia and tachypnea. Initially, in Class II, there is orthostatic hypotension followed by persistent hypotension in the prone position after further blood loss.

The best way to manage a patient who is bleeding is to do what the body does, and that is to infuse fluids into the vascular space in the form of crystalloids. Patients can tolerate the loss of 75 percent of the circulating red cells as long as the vascular volume is maintained. However, uncompensated loss of vascular volume >30 percent (Class III–IV) may be fatal. Therefore, red cells rarely need to be transfused until the response of the patient to rapid infusion of 2,000 ml of crystalloid can be assessed.

Most trauma centers use crystalloid rather than colloid as the initial replacement fluid for patients in hemorrhagic shock. A meta-analysis of several studies comparing crystalloid to colloid concluded that mortality was higher in trauma patients who initially
received colloid compared to those who received crystalloid. Their interpretation was that trauma leads to a pulmonary capillary leak syndrome; and under these circumstances, albumin can “leak” into the alveolar spaces predisposing to pulmonary edema. On the other hand, in non-trauma patients with an intact pulmonary vasculature, colloid may be a better initial replacement solution. A recent study from Australia with almost 7,000 patients, however, showed no difference in outcomes using 4 percent albumin versus normal saline.

When crystalloid is administered about 1/3rd goes to replace the extracellular fluid that initially entered the vascular space, 1/3rd of it is excreted to maintain urine output, and 1/3rd stays in the vascular space. Therefore, for crystalloid to adequately replace lost blood, about 3 X the volume of blood loss is required (the “three for one” rule). There may, however, be situations where complete restoration of vascular volume is not desirable until the patient is in a facility where the source of blood loss can be adequately controlled, presumably because of the danger of disrupting newly formed thrombi at bleeding sites.

STORAGE LESION OF BLOOD COMPONENTS

One of the main management problems associated with MT arises because of the “storage lesion” developing when cellular components are stored for periods of time. With small volumes of blood transfusion, the storage lesion does not produce any clinical findings. The “storage lesion” develops because the cellular components change during storage, the materials used for storage can lead to physiologic effects, or there are biochemical changes that occur. The storage lesion changes are listed in Table 14.2 and each will be described in detail.

Table 14.2: The storage lesion

- Citrate effect
- Acid-base changes
- Alteration in hemoglobin function
- Loss of platelet function
- Dilutional coagulopathy
- Alteration in potassium level

Citrate Effect

Citrate is the anticoagulant used in most blood components. Thus, in an MT situation, a large amount of citrate may be given to an individual. Normal individuals can metabolize citrate rapidly (equivalent to the amount of citrate in one unit of blood every five minutes). However, since citrate is metabolized in the liver, individuals with either liver disease or impaired hepatic circulation, may have excessive accumulations of citrate. Citrate alone is not toxic, and the effects of citrate depend either on the ionized calcium or on the magnesium-binding properties of citrate. Thus, the most serious effect of excess citrate is hypocalcemia. Many factors contribute to the ionized calcium levels in patients receiving citrate including acid-base balance and the rate of mobilization of calcium from skeletal stores. Patients undergoing plasma exchange have rapid and significant increases in serum parathormone levels which obviously would influence calcium mobilization. Ionized calcium varies with alteration in pH, being decreased with alkalosis and increased with acidosis. Because the level of ionized calcium is impossible to predict in an MT situation, routine administration of calcium should be discouraged. Rather, calcium administration should be based on evidence of hypocalcemia, either clinically or by frequent laboratory measurement of ionized calcium. Serious or fatal physiologic effects are seen with both hypocalcemia and hypercalcemia.

As stated above, citrate also binds magnesium, as well as calcium. There have been occasional reports of hypomagnesemia occurring in MT situations. In a couple of cases, the hypomagnesemia was felt to contribute to death.

Acid-Base Balance

The metabolic activity of red cells during storage produces increasing amounts of hydrogen ion. Therefore, stored red cell units become progressively acidotic. This acid load in stored blood theoretically could produce a serious acidosis in massively transfused patients. However, studies of casualties occurring in the Vietnam War show that this acid load is very easily handled in individuals who have normal blood volumes and normal functioning livers and kidneys. On the other hand, patients whose blood volume cannot be restored do develop a progressive acidaemia. This acidemia is more likely due to the persistent shock rather than to the transfusion of acidotic blood.
Perhaps, one reason that acidosis generally does not occur with the infusion of a large acid load is that the accompanying citrate is metabolized in the liver to bicarbonate, which counterbalances the acidosis. Patients receiving a liver transplant generally receive multiple units of platelets and fresh frozen plasma (FFP) containing large amounts of citrate during this transplantation and become alkalotic shortly after the new liver is in place, demonstrating the importance of the liver in the metabolism of citrate. Also, patients receiving MT will frequently show a rebound alkalosis after the episode is completed due to the bicarbonatic accumulation. Alkalosis is known to have several adverse physiologic affects, including detrimental left shift to the hemoglobin oxygen association curve (to be discussed later), hypocalcemia, and hypokalemia. Therefore, alkalosis should be avoided when possible. In particular, exogenous alkali should not be administered in an effort to reverse acidosis as long as the patient’s blood volume can be rapidly restored. For these reasons, careful laboratory monitoring of the pH should dictate when extra alkali is needed therapeutically.

**Hemoglobin Function**

Red cells function because their hemoglobin picks up oxygen in the lungs and carries this oxygen to the tissues, unloading the oxygen at the appropriate times so the tissues may use this oxygen for metabolism. The ability of hemoglobin to offload oxygen at the tissue level depends on there being normal amounts of 2,3-diphosphoglycerate acid (2,3-DPG) in the red cells. Unfortunately, in spite of improved storage media, red cells lose 2,3-DPG with storage so that by day five to seven of storage, red cells have significantly reduced 2,3-DPG levels. This loss of 2,3-DPG results in a left shift in the hemoglobin oxygen dissociation curve resulting in tighter affinity of hemoglobin for oxygen and more difficulty offloading the oxygen to the tissues. Thus, the $P_{50}$ value of transfused red cells is decreased. Theoretically, then, red cells transfused in an MT situation should be less than seven days old) do better than patients who get blood of variable storage times.

Why is this discrepancy seen between the theoretical problems associated with shifting the hemoglobin dissociation curve to the left and the actual clinical results? The primary reason is probably that 2,3-DPG levels are rapidly replenished in the red cells after transfusion (in the first hour or two). Another reason is that the hemoglobin oxygen dissociation curve shifts rapidly and responds to environmental situations, in particular altered temperature and pH. Acidosis rapidly produces a favorable shift of the hemoglobin oxygen dissociation curve to the right resulting in improved oxygen delivery. As seen above, acidosis to some degree is usually present early in patients requiring MTs. Alkalosis, as stated before, is associated with a detrimental left shift to the hemoglobin oxygen dissociation curve resulting in difficulty offloading oxygen to the tissues. This is a significant reason why alkalosis needs to be avoided in MT.

**Coagulation Abnormalities**

Some of the coagulopathy seen in association with MT occurs because of the loss of coagulation function with storage. For example, if a unit of whole blood is stored at 4°C, all of the platelet function will be lost within 24 hours and there will be a significant drop in Factor V and Factor VIII within a week or so. For this reason, in order to maintain platelet function, platelets are stored at room temperature and plasma is stored in a frozen state in order to prevent decline in coagulation factor activity.

The thrombocytopenia associated with MT is often one of the earliest manifestations of coagulopathy. This thrombocytopenia is due to multiple factors including delutional thrombocytopenia, disseminated intravascular coagulopathy (DIC), and consumptive coagulopathy due to utilization of platelets at bleeding sites. Generally, however, thrombocytopenia is not observed until about 1½ to 2 blood volumes have been replaced. With stress, the body has a significant ability to mobilize platelets from storage sites in the spleen and to more rapidly release platelets from bone marrow. In MT, platelet counts generally should be maintained in the range of 50 to $100 \times 10^9/L$. In order to do this and to time platelet transfusions, frequent determinations of platelet count need to be done.
As stated above, Factors V and VIII decline in stored blood at 4°C. However, most red cells now are transfused using extended storage media in which most of the plasma has been removed. Therefore, most red cell transfusions are given without much plasma associated with them. Because of the lack of significant plasma coagulation factors in red cells, coagulopathy associated with the loss of coagulation factors results in both an elevation of the prothrombin time (PT) and the partial thromboplastin time (APTT). In patients receiving red cells stored at extended storage media, one-third of patients receiving less than 12 units of blood and all patients receiving more than 12 units of blood had elevations of PT and APTT greater than 1.5 × normal. Severe thrombocytopenia (<50 × 10^9 /L) was seen in all of the patients only after 20 units of blood. Generally, an elevation of the PT and/or the APTT greater than 1.5 × normal is felt to be an indication for the use of FFP.

As stated above, coagulopathy seen in MT is multifactorial. Generally, laboratory values do not allow distinction between dilutional coagulopathy, DIC, and consumptive coagulopathy due to multiple bleeding sites. In DIC, hypofibrinogenemia is often seen early in the process. In the author’s experience, this is often an early finding in patients with marked bleeding due to trauma. Therefore, frequent monitoring of PT, APTT, fibrinogen and platelet levels are necessary to give appropriate replacement therapy. For decreased fibrinogen levels, cryoprecipitate is the component of choice.

The coagulopathy associated with MT is very difficult to reverse once it becomes established. Therefore, monitoring coagulation studies in the laboratory frequently becomes very important so that the appropriate therapy can be started as soon as an early coagulopathy is identified. The guidelines for administration of components in our institution will be given at the end of the chapter.

Hypothermia is a frequent clinical finding in patients with MT. Hypothermia induces a significant platelet dysfunction and, therefore, contributes to the coagulopathy of MT. This platelet dysfunction, however, is completely reversible with restoration of normal body temperature. Therefore, multiple techniques, including adequate covering of the patient and infusion of blood and components through inlying blood warmers, need to be adopted in managing patients with MT. Besides the coagulopathy associated with hypothermia, serious cardiac arrhythmias can also occur.

**Potassium**

Potassium progressively leaks from stored red cells into the storage medium and, therefore, levels of potassium in the liquid around the red cells can be quite high. For this reason, there obviously would be concern about hyperkalemia following transfusion of large amounts of red cells. However, since red cells very rapidly restore their intracellular potassium concentrations after transfusion, predicting the effect of MT on serum levels of potassium is impossible. Both hyperkalemia and hypokalemia have been observed in some patients following MT.

In these studies, there was no correlation between the volume of transfusion and the level of potassium in the serum. Therefore, potassium levels need to be monitored carefully and appropriate intervention instituted when necessary.

**IMMUNOSUPPRESSION**

The immunosuppressive effect of blood transfusions was identified many years ago when investigators recognized that renal allografts survived longer in recipients who had previously received red cell transfusions than in recipients who had never been transfused. Since the immunologic response of the body to allografts and to cancers have many similarities, this observation leads to speculation that perhaps transfusions might adversely affect the ability of individuals to control their malignancies. During the last 20 years, there have been many studies looking at whether transfusions led to increased cancer recurrence in certain patients. A few of these studies did show a slight increase in the relative risk of cancer recurrence in patients with selective cancers. However, evaluation of all of these studies have led to the conclusion that there is no definite evidence of an important deleterious effect of blood transfusions on cancer immunosurveillance.

On the other hand, there are numerous studies showing an increase in infections following transfusions. In over 30 studies, blood transfusions were associated with up to a 10-fold increase in the incidence of infections following the transfusion
episodes. The mechanism for this increased risk of infection might relate to the fact that transfusions induce a shift in T-helper function from Type 1 to Type 2. Therefore, TH2 cytokines are increased and TH1 cytokines are decreased. This results in impaired monocyte and natural killer function and reduced phagocytosis. Therefore, microorganisms are not handled as well in patients who have received transfusions.

Though there are some conflicting results in some of the studies, in particular in relation to the effect of transfusions on cancer recurrence, there definitely appears to be a significant immunosuppressive effect following blood transfusion. This immunosuppressive effect has been referred to as transfusion-associated immune modulation (TRIM). The studies that do show evidence of TRIM demonstrate a definite dose response to the amount of blood transfused. Therefore, patients receiving MT are particularly at risk for this TRIM and any minor immune suppression from a few units of blood would be magnified in patients receiving MT. Most of the studies demonstrate that leukoreduction decreases TRIM, but does not completely eliminate it. Therefore, immune suppression associated with MT might be reduced when universal leukoreduction is adopted.

NEW TREATMENTS: FACTOR VIIA

Recombinant Factor VIIa was initially developed for treatment of patients with hemophilia who have inhibitors to Factor VIII. Factor VIIa functions by inducing the “thrombin burst” usually generated by Factor VIII and Factor IX on the surface of activated platelets.

Recently, several investigators have used recombinant Factor VIIa in MT episodes in which the coagulopathy does not appear to be corrected with standard methods of replacement. In such situations, presumably, the high dose of Factor VIIa can directly bind to activated platelets and induce the thrombin burst necessary to produce enough fibrinogen interacting with activated platelets to induce a satisfactory hemostatic clot. After Factor VIIa therapy, the prothrombin time is always normal or shortened. This correction of prothrombin elevation may not necessarily always correlate with adequate hemostasis. The dosage in massive transfusion is somewhere between 50 and 90 mcg/K. This can be repeated within a few hours if bleeding is not brought under control.

To date, there are no well-performed controlled studies documenting the value of Factor VIIa in MT. Almost all of the reports in the literature are in the form of case reports. Though the reports generally show beneficial effects, Factor VIIa needs to be used with caution since there are a few reports of thrombosis and/or embolic episodes associated with Factor VIIa treatment. In particular, this drug should be used with caution in patients who have underlying conditions predisposing them to thrombosis and DIC, including atherosclerotic diseases, advanced age, and crush injury. Most reports emphasize that Factor VIIa only be used after standard methods to correct the coagulopathy have been ineffective. Furthermore, at the present time this drug is quite expensive.

RED CELL SUBSTITUTES

For many years, investigators have explored the possibility of providing an oxygen carrying and off-loading molecule, which could avoid the necessity of having compatible red cells available for transfusion. The most promising of these investigations have centered around free hemoglobin solutions. Initial research concentrated on tetrameric hemoglobin, which was found to have a number of complications including short intravascular retention, decreased affinity for oxygen, low osmotic activity, and significant vasoconstrictive properties.

Subsequent investigations have concentrated on the use of polymerized hemoglobin of various types. These products have proven to be significantly better products than tetrameric hemoglobin since they have a longer half-life, and they have less vasoconstrictive properties. Currently, there are three polymerized hemoglobin products undergoing testing for the Food and Drug Administration (FDA) approval in the United States. These products are listed in Table 14.3.

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Polymerization method</th>
<th>Hemoglobin source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HemoLink</td>
<td>Hemosol Inc.</td>
<td>Alpha-Raffinose</td>
<td>Human</td>
</tr>
<tr>
<td>HemoPure</td>
<td>BioPure Corp.</td>
<td>Guterallyhyde</td>
<td>Bovine</td>
</tr>
<tr>
<td>PolyHeme</td>
<td>Northfield Lab</td>
<td>Guterallyhyde</td>
<td>Human</td>
</tr>
</tbody>
</table>
To date, PolyHeme has undergone the most trials and is in phase III clinical trials at the present time. It actually has been approved for replacement of acute blood loss in South Africa. In patients where this product has been used, the red cell hemoglobin concentration can be reduced quite significantly while the total hemoglobin concentration can be maintained adequately with PolyHeme and adequate oxygenation can be achieved. As with all of the red cell substitutes, the half-life of PolyHeme is relatively short and it does have a somewhat increased P50 level which decreases offloading of oxygen. Therefore, this product can be used only as an interim solution to transfusion.

Because of the above studies, there is a high likelihood that PolyHeme will be approved shortly by the FDA for use in trauma situations. Since this product is universally compatible and has a long shelf-life, its use seems logical in situations where red cell transfusions are not immediately available. It has been shown to save lives under these circumstances. Following the infusion of PolyHeme, patients can be transported to facilities where red cell transfusions are available or red cells can be brought to the facility caring for patients who have undergone marked hemorrhage.

**INTRAOPERATIVE AUTOLOGOUS TRANSFUSION**

Many intraoperative autologous transfusion systems have been introduced into clinical care in the past 20 years. Most of these are quite effective at salvaging and reinfusing red cells at the time of bleeding during surgery. The transfusion medicine specialist managing a patient with MT, however, needs to be aware that intraoperative autologous transfusion being used. This is because almost all of these systems wash the red cells in saline prior to reinfusing them. Since the transfusion specialist is only monitoring the amount of allogeneic blood being given to the patient, there will be an underestimation of the amount of transfusion needed by that patient. Because no coagulation proteins nor platelets are reinfused with intraoperative red cell transfusions, the need for component replacement and support may be greater than expected. Therefore, it is critical that the transfusion medical specialist know that autologous intraoperative transfusion is being given and have some idea of the volume of reinfused blood.

**SURVIVAL AFTER A MASSIVE TRANSFUSION**

Frequently, care givers providing support to a patient with MT will question how far they should go with support. There are two recent studies looking at the survival of patients receiving greater than 50 units of red cells and these studies demonstrate a 43 percent and a 45 percent survival. One study showed clearly that neither the total blood product requirement nor the type of components used were identified as independent risk factors associated with morality. Therefore, continued component therapy should be maintained despite massive transfusion and the decision whether a patient can survive a severe hemorrhagic episode should be based on factors other than the amount of blood and blood components received.

**MASSIVE TRANSFUSION PROTOCOL**

For many years, our institution has had a massive transfusion protocol which is initiated by the technologists in the Blood Bank. Once a patient using four or more units of red cells within one hour with a projected continued use is identified, the Medical Technologist notifies the Transfusion Service Director. This person then consults with the clinicians taking care of the patient whether in the intensive care unit (ICU) or in the operating room. He or she assumes responsibility for the administration of components based on frequent laboratory tests outlined in Table 14.4.

<table>
<thead>
<tr>
<th>Table 14.4: Monitoring massive transfusions by lab testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Baseline tests</td>
</tr>
<tr>
<td>• Repeat baseline tests plus</td>
</tr>
<tr>
<td>• FDP after the first 4 units are transfused;</td>
</tr>
<tr>
<td>• Repeat Hgb, Plt, PTT, APT, and fibrinogen every additional 6–10 units of RBCs</td>
</tr>
<tr>
<td>• After every 10 units of RBCs Ca++, Mg++, pH, and lactate</td>
</tr>
</tbody>
</table>

The Transfusion Service Director can assure that laboratory tests are done in a timely and efficient manner. Utilizing these laboratory tests, he or she recommends the following management to the clinicians (Table 14.5).
Table 14.5: Guidelines for managing blood component therapy during massive transfusion

<table>
<thead>
<tr>
<th>Component</th>
<th>Lab results</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFP</td>
<td>APTT 50 seconds and/or PT&gt;18 seconds (1.5 × normal)</td>
</tr>
<tr>
<td>Platelets</td>
<td>Platelet count&lt;100,000 μl</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>Fibrinogen &lt;150 mg/dl</td>
</tr>
<tr>
<td>Dosage of components varies depending on the rate of development of coagulopathy and severity</td>
<td>Generally starts with:</td>
</tr>
<tr>
<td></td>
<td>2–4 units FFP</td>
</tr>
<tr>
<td></td>
<td>6 units of platelets</td>
</tr>
<tr>
<td></td>
<td>8 units of cryoprecipitate</td>
</tr>
</tbody>
</table>

The Transfusion Service Director continues involvement with a case until the patient becomes stable. The best way to monitor the patient is on a flow sheet, which records the laboratory data and the amount of components used.

**SUMMARY**

As discussed above, the Transfusion Service Medical Director plays a major role in the management of patients undergoing MT. In our institution, this role is welcome because the clinicians can be free to tend to other serious matters in patient’s undergoing massive hemorrhage. Our experience has been that if a patient has near normal coagulation tests at baseline, the development of a serious coagulopathy during the procedure can be avoided, no matter how much blood is used. The Transfusion Service Director, therefore, plays a very important role as liaison between the laboratory and clinical environment in which the patient is being managed.28

**REFERENCES**

Beta-thalassemia major and sickle cell anemia are the most common inherited hemoglobinopathies that require regular long-term blood transfusions as part of routine management. Although blood transfusion therapy is an established management strategy in beta-thalassemia major with benefits outweighing the risks, its value as therapy in sickle cell disease (SCD), is still unclear with regards to benefits versus risks. This is especially so in an era where the life expectancy in both these diseases is progressively increasing. This review will focus on the recent advances on the guidelines regarding safe blood transfusion practice in the treatment of beta-thalassemia major and SCD with an emphasis on risks versus benefits of transfusion therapy.

PRINCIPLES OF BLOOD TRANSFUSION IN SICKLE CELL DISEASE

Sickle cell disease (SCD) is a genetic hemoglobin disorder which causes painful crises and dysfunction of virtually every organ system in the body, ultimately leading to premature death. It is predominantly seen in the sub-Saharan Africa, India and parts of the Mediterranean, but population movement has made this a worldwide problem. Approximately 60,000 Afro-Americans, 10,000 people in the UK and one in sixty people in West Africa now suffer from the disease.\(^1\)\(^2\) Despite adequate care and facilities for sickle cell patients in developed countries, the average life expectancy for men and women with homozygous disease (SS) is still only 42 and 48 years respectively.\(^3\)

In SCD, under certain hypoxic conditions, the hemoglobin molecules within the red blood cells can associate as polymers, making the cells rigid and distorted into a variety of shapes, some resembling a sickle. The red blood cells have a shortened lifespan, resulting in anemia. Sickle red cells express surface adhesion molecules causing them to stick to the cells lining the blood vessels and block them.\(^4\) In later life, chronic damage to poorly perfused organs becomes apparent.\(^5\) The clinical spectrum of this disease is variable, commonly characterized by repeated acute painful episodes, increased vulnerability to infections, and certain pathological phenomena such as acute chest syndrome, cerebrovascular strokes, hepato-pathy, and multiorgan failure. Individual heterogeneity between patients make the symptoms of the disease highly variable in frequency and severity, but the most pathognomonic manifestation is the acute painful crisis which occurs when small vessels are blocked, depriving the tissues of oxygen and causing ischemic damage and pain. Vaso-occlusion can also occur in some large vessels, such as those in the brain, causing stroke.

Stroke, usually ischemic, occurs in up to 10 percent of children with sickle cell anemia\(^6\) and can cause slurring of speech, weakness in the limbs, coma and cognitive impairment. ‘Silent infarctions’ often go unnoticed but can also cause significant neurological damage and disability and are reported to be present in a further 17 to 25 percent of patients.\(^7\) Further strokes (secondary stroke) occur in a half to two-thirds of untreated patients and are associated with increasing morbidity and mortality.\(^6\)

As risk factors for first stroke are not well established, the focus to date has largely been on secondary prevention. However, with the advent of transcranial Doppler screening studies, high blood
flow in one or more major arteries indicating vessel narrowing, has been demonstrated to predict for an increased risk of stroke; and, therefore, preventative treatment could even be started prior to first stroke.8,9

Blood transfusions are undertaken in many patients to dilute the circulating sickle cells, thus reducing the risk of vaso-occlusive episodes and anemia10 and increase tissue oxygen delivery. Transfusions can be given acutely, in emergency treatment of complications such as acute splenic sequestration and acute chest syndrome, and are also frequently used in preparation for surgery. In addition, many patients have chronic transfusion regimes in an attempt to prevent severe vaso-occlusion and stroke.6 However, the relative risks and benefits of blood transfusion programs are not fully defined and vary due to a number of factors.

Allogeneic red cells collected from volunteer blood donors according to Food and Drug Administration (FDA) regulations are appropriate for patients with SCD. The shelf-life of the blood (time since collection) is usually not important as long as it is in date (i.e. before expiration date). Exchange transfusion with blood less than 10 days old (less than 5 days in infants) helps in acute situations requiring immediate correction of the oxygen-carrying capacity. Transfused blood should be screened for the presence of Hb S and confirmed to be negative. This procedure eliminates blood from donors with sickle trait and, hence, allows the accurate measurement of the percentage of Hb S, post-transfusion. It is advisable that the RBC antigenic phenotype of all patients older than 6 months be determined and a record of the results be maintained in the blood bank. Phenotype determination should include, at least, the ABO, Rh, Kell, Kidd, Duffy, Lewis, Lutheran, P and MNS blood groups. All patients with a history of prior transfusion should also be assessed for the presence of alloantibodies.

Prestorage leukocyte depletion of red cells is standard practice to reduce febrile reaction, platelet refractoriness, infections, and cytokine-induced complications and are recommended for transfusion,11 especially in children, because they reduce febrile reactions, decrease alloimmunization to leukocyte antigens and minimize the transmission of cytomegalovirus (CMV). Leukoreduction can be satisfactorily achieved at the bedside using fourth-generation in-line filters. Washed RBCs should be used in patients who have a history of severe allergic reactions following prior transfusions. Children who are candidates for bone marrow transplantation should also receive irradiated cellular blood products and relatives should not be used as blood donors.

Indications for Transfusions

The lifespan of sickle cells is only 15 to 20 days compared to 120 days for normal red cells; and as a result of this, most patients with SCD have moderate anemia.12 This may be exacerbated further in the event of complications such as splenic sequestration crisis, hyperhemolysis, infection and aplastic crisis. Transfusions are used to raise the oxygen-carrying capacity of blood and decrease the proportion of sickle red cells. Clinically, they will also improve microvascular perfusion to the tissues and can potentially limit or decrease the degree vaso-occlusive crisis.12

Transfusions fall in two categories;

**Acute:** Episodic transfusions to stabilize or reverse complications;
**Chronic:** Prophylactic transfusions to prevent future complications.

**Acute Transfusions**

a. **Severe anemia:** Simple transfusions should be considered without removal of any blood in patients:
   i. who are so anemic that they have physiological derangement, manifested by impending or overt high output cardiac failure, dyspnea, postural hypotension, angina, or cerebral dysfunction.
   ii. who have had a sudden reduction in hemoglobin concentration, particularly patients having an acute splenic or hepatic sequestration crisis, manifested by rapid splenic or liver enlargement and rapidly falling hematocrit.
   iii. who exhibit fatigue and dyspnea, usually at hemoglobin concentrations less than 5.0 g/dl and hematocrit less than 15 percent in association with erythroid hypoplasia or aplasia.
   iv. who exhibit hyperhemolysis in association with infections, especially acute chest syndrome and malaria.

The aim of such transfusion therapy needs to be individualized.13 In general, phenotypically matched, sickle negative, leukodepleted packed
red cells is the blood product of choice with the view to limit the post-transfusion hematocrit to around 36 percent or less to avoid complications of hyperviscosity in SCD patients.

b. **Sudden severe illness:** Transfusions can improve tissue oxygenation and perfusion and are indicated in seriously ill patients to potentially limit areas of vaso-occlusion in certain life-threatening events with the aim of therapy to maintain the Hb S level below 30 percent.

i. Acute, impending, or suspected cerebrovascular accidents, including transient ischemic attacks.

ii. Acute chest syndrome including “fat embolization.”

iii. Severe sepsis.

iv. Acute multiorgan failure.

c. **Preoperative:** There is evidence to recommend transfusions to sickle cell patients before major surgery. Anemia should be corrected to the Hb concentration of about 10 g/dL and the Hb S below 60 percent. Patients randomized to a more aggressive regimen to reduce the Hb S below 30 percent did not show any significant reduction in perioperative complications.

d. **Pregnancy:** Pregnancy carries some increased risk for women with SCD and for her fetus. The rate of fetal loss is high, presumably because of placental infarcts. Miscarriage and preterm labor are also more common than in women who have normal types of hemoglobin.

Sickle cells have a shorter lifespan than do normal red blood cells, and it has been suggested that the only consistently successful way of reducing the incidence of complications is by regular blood transfusions to maintain the proportion of normal hemoglobin at 60 to 70 percent of the total. This is said to both dilute the circulating sickle hemoglobin and also raise the hemoglobin level and reduce the stimulus to the bone marrow to produce more sickle hemoglobin. However, there are no large controlled studies to evaluate this premise.

**Chronic Transfusions**

Chronic blood transfusions are indicated to avoid potentially serious medical complications despite the risks of alloimmunization, infection and iron overload. The aim is to maintain the Hb S level between 30 and 50 percent. This can be achieved by simple transfusions or preferably by red cell pheresis or exchange transfusions to reduce the risk of iron overload.

i. Primary prevention of stroke and prevention of stroke recurrence.

ii. Chronic debilitating pain.

iii. Pulmonary hypertension.

iv. Anemia associated with renal failure.

v. Chronic heart failure.

**Equivocal Indications**

Under certain conditions, the current practice of some institutions is to perform a red cell exchange for certain conditions; however, there is no clear-cut evidence whether this practice is either useful or necessary. These conditions are the following:

i. Minor surgical interventions needing general anesthesia for a short time, e.g. endoscopy.

ii. Intractable acute events.

iii. Acute priapism unresponsive to therapy.

iv. Surgery on the posterior segment of the eye, even when done under local anesthesia in a nonanemic patient. Transfusion is not needed for laser surgery.

v. Management of silent cerebral infarcts detected by magnetic resonance imaging (MRI) or defects with neurocognitive defects.

vi. Before injection of contrast material. However, newer agents like gadolinium and nonionic contrast media have a considerable lower risk.

vii. Leg ulcers.

viii. The presence of several chronic conditions related to sickle cell disease.

In all these cases, the proportion of normal cells circulating should be maintained above 70 percent by repeated simple transfusions.

**Nonindications**

The following are not considered appropriate indications for transfusion, and its use is not recommended in these clinical settings:

i. **Chronic steady-state anemia:** Most patients with sickle cell disease are relatively asymptomatic and do not require transfusions to improve oxygen-carrying capacity.
ii. Uncomplicated painful episodes.
iii. Minor infections.
iv. Minor surgery not requiring prolonged general anesthesia (e.g. myringotomy, simple biopsy).
v. Aseptic necrosis of the hip or shoulder (except when surgery is required).

**Transfusion Methods**

**Simple Transfusions**

Simple transfusions can be used for acute anemia or hypovolemia or in a chronic transfusion program. Packed red cells should not be used when volume expansion is needed. The post-transfusion hematocrit should be 36 percent or less to prevent hyper-viscosity.  

**Exchange Transfusion**

Exchange transfusion is used to rapidly alter the hemoglobin level and replace sickle cells with normal erythrocytes. This type of transfusion reduces the concentration of sickle cells without substantially increasing the hematocrit or whole-blood viscosity. Several methods are available that achieve this purpose. The volume needed can be calculated from the patient’s weight, initial hematocrit, target hematocrit, and desired percentage of Hb A. An adult exchange usually takes about 6 to 8 units and children require about 50 to 60 ml/kg of blood. Whole blood or packed cells reconstituted to hematocrit of 30 to 40 percent are used. In adults, blood can be removed as 500 ml bags followed by infusion of 500 ml reconstituted blood and repeated to achieve the final Hb A concentration as desired in the individual patient.

**Recommended Manual Scheme**

1. Bleed one unit (500 ml) of blood from patient.
2. Infuse 500 ml of saline.
3. Bleed a second unit from the patient.
4. Infuse two units of blood.
5. Repeat above steps as necessary.

In children, and in adults with poor venous access, smaller volumes will be obtained and the above scheme should be suitably modified.

**Rapid Partial Exchange**

In some patients, whole blood can be removed from one arm at the same time that donor cells are transfused into the other arm. In adults, this procedure can be performed in 500 ml units. In children, the individual exchange aliquots are adjusted to a safe and practical level. The total volume of blood to be used is proportional to the patient’s body weight and hematocrit; thus, different formulae are needed for different initial hematocrit ranges. Exchange transfusions performed with whole blood (or packed cells reconstituted to the volume and hematocrit of whole blood using saline or other diluents) are more efficient than those using donor packed cells. They may reduce the number of units needed but take slightly more time.

**Automation**

Automated full red cell exchange with the use of continuous or discontinuous flow instrumentation is very rapid, effective and safe. It uses packed red cells but may be difficult in small children because of vascular access problems. Successful red cell exchange with a discontinuous flow system is possible, but the red cell concentration must be diluted in the bowl of the apparatus. The advantage of this procedure is that it prevents iron overload, but venous access and cost are the limiting factors preventing its widespread use.

**Subacute Partial Exchange Transfusion**

Often, it may be more convenient to reduce the level of hemoglobin over a period of days rather than acutely. If additional reduction of Hb S is needed, further exchanges should be performed using whole blood and not packed red cells.

**Chronic Transfusion Programs**

Once a sufficient level of transfused normal cells (>70% Hb A) is achieved, it is often useful to maintain this for a period of weeks to years for proper indications. This proportion of normal cells can be maintained by simple transfusions at intervals of 2 to 4 weeks. The levels of Hb A and Hb S must be monitored by quantitative hemoglobin electrophoresis. Suppression
of reticulocytosis by transfusion is an appropriate goal. However, iron overload is inevitable.

**Transfusion Complications**

Transfusion complications for sickle cell patients are as those for any patient receiving acute or chronic transfusion. Interestingly, transfusions have precipitated painful episodes, strokes, and acute pulmonary insufficiency under certain circumstances.\(^\text{12,20}\)

**Volume Overload**

This occurs when too much volume is transfused too quickly. This is especially important in patients who have cardiac dysfunction or poor cardiac reserve. Administration of intravenous diuretics, partial removal of red cell supernatant fluid before transfusion and slow infusion rate of 2 to 4 ml/kg/hr will help reduce this problem.\(^\text{20}\)

**Iron Overload**

There is no simple test to determine iron overload. Measurement of serial serum ferritin may help but is unreliable as ferritin being an acute phase reactant is elevated in liver disease, inflammation, etc. Liver biopsy is the most accurate test for assessing iron overload but is an invasive procedure. Moreover, multiple procedures are necessary to monitor iron overload status. Chelation is recommended if the liver iron exceeds 7 mg/g dry weight. Liver iron can be assessed by two noninvasive methods namely superconducting quantum interference device and MRI. The former is available only in few centers over the world and the latter needs to be standardized for universal usage. Alternatively, cumulative transfusions of 120 cc of pure red cells per kg of body weight can be used to initiate chelation therapy.\(^\text{18}\)

All multiply transfused patients should have serum ferritin levels measured periodically. If the level exceeds 2,500 ng/ml and transfusions are still required, patients should be considered for chronic chelation therapy using desferroxamine. It needs to be initiated at the dose of 25 mg/kg/day as an 8 hourly subcutaneous infusion.\(^\text{22}\) The dose and durations should be individualized. Supplementation of ascorbic acid at 100 to 200 mg daily can increase chelation, if the patient is vitamin C deficient. Where possible, urinary iron excretion after test doses of desferroxamine (20 mg/kg and, if necessary, 40 mg/kg) should be measured. A difficulty with this therapy, which requires repeated subcutaneous infusion of medications, is patient compliance. Ongoing education and support, often provided by a specially trained nurse, is usually necessary to maintain the patients’ cooperation.

**Alloimmunization to Red Cell Antigens**

The incidence of alloimmunization to red blood cell antigens in transfused patients with sickle cell anemia is approximately 20 to 25 percent, which is greater than in the general population.\(^\text{23}\) This condition causes difficulty in obtaining compatible blood and results in a high incidence of delayed hemolytic transfusion reactions.\(^\text{24,25}\) The delayed transfusion reaction occurs 5 to 20 days after transfusion and is due to antibodies not detectable at the time of compatibility testing despite sensitization of the recipient. It has been found that 30 percent or more of the antibodies to red cell antigens may disappear with time, although the recipient is capable of mounting an anamnestic response to further stimulation. The delayed hemolytic transfusion reaction may result in severe anemia, onset of painful crisis, or even death.

Alloimmunization and hemolytic transfusion reactions resulting from it can be reduced by the following:

i. Acquiring and maintaining adequate records of previous transfusions and complications arising from them.

ii. Limiting the number of transfusions administered.

iii. Screening for newly acquired antibodies 1 to 2 months after each transfusion to detect transient antibodies capable of causing a subsequent delayed reaction.

iv. Diminishing the opportunities for alloimmunization because of mismatch in the antigens of donors and patients.\(^\text{26}\)

v. Typing the patient before transfusion (if this has not already been done) for antigens of the Rh and Kell blood groups and avoiding the transfusion of cells bearing these antigens (particularly E, C, and Kell) if the patient lacks the antigen.

vi. More complete antigen matching has been suggested, but it is expensive, and the utility of such matching is not clear.
vii. Increasing the use of ethnic donors of blood because of the similarity of red cell antigenic phenotypes. Family members and community groups can assist in accomplishing this objective.

The patient alloimmunized to one red cell antigen is more likely to become alloimmunized to others, and care should be taken in selecting transfusion units. Transfusions should be given only for clear-cut indications. These patients should be counseled to advise any new physician of their history of alloimmunization. Carrying a card or an identification bracelet with the red cell phenotype and any identified antibodies listed on it is strongly recommended.

**Autoimmune Anemia following Allosensitization**

In some highly alloimmunized patients, a syndrome of autoimmune hemolytic anemia may follow allosensitization or a hemolytic transfusion reaction. In this case, the patient may become more anemic than before transfusion and the direct antiglobulin (Coombs’) test remains positive even after the incompatible transfused cells have been destroyed. This syndrome is due to the making of antibodies directed against self-antigens and may persist for several weeks to 2 to 3 months before disappearing. Further transfusion is complicated by the autoimmune antibody and requires sophisticated blood banking techniques to find “least incompatible” blood for transfusion. Although transfusion may be necessary in some patients, an alternative course may be to avoid transfusions and to administer corticosteroids, large doses of erythropoietin, and possibly intravenous immunoglobulins.

**Infection**

Hepatitis and other transfusion-transmitted viral diseases in blood occur with the same frequency in sickle cell patients as in other patients receiving transfusions. The effects may be more severe in sickle cell patients because of the presence of the disease.

Post-transfusion HIV infection and AIDS are reported in sickle cell disease, occurring as late as 5 to 8 years after the transfusion with blood not known to be from an infected donor. Thus, patients with sickle cell disease who were transfused before blood products were tested for HIV antibodies (1975–1985) as well as those transfused with today’s “safe” blood should be considered for counseling on testing for HIV infection.

Parvovirus occurs in 1 in every 40,000 units, and is associated with acute anemic events and multiple sickle cell complications.

Transfusion-induced bacterial infections are uncommon. Repeatedly transfused hemoglobinopathies patients are particularly vulnerable to *Yersinia enterocolitica* and bacteremia from poor skin cleansing before phlebotomy. All patients who develop fever after transfusion need to be assessed immediately for potential bacterial infections.

**Alternatives to Blood Transfusion**

Among the alternatives to blood transfusion, erythropoietin (EPO) and blood substitutes have been tried in a small number of patients with SCD with some success. Blood substitutes may play a role in the short-term management of alloimmunized patients and in preparation for surgery. Serum levels of EPO in adult patients with SCD are lower than in adults with other hemolytic anemias. Renal insufficiency and the decreased oxygen affinity of Hb S may contribute to the inappropriate levels of serum EPO. Large doses of EPO (especially in patients with serum levels of EPO <500 μg/ml) in association with supplemental iron therapy (even in iron-overloaded patients) and hydroxyurea may be helpful in selected patients. This approach to therapy in patients with SCD, however, requires further evaluation in controlled trials.
PRINCIPLES OF BLOOD TRANSFUSION IN BETA-THALASSEMIA MAJOR

Thalassemia syndromes are a family of genetic blood disorders characterized by an imbalance in the synthesis of globin chains, which may result in the absence or reduction of the production of adult hemoglobin. The major component found in adult red blood cells is hemoglobin A, which consists of two alpha and two beta protein ‘globin’ chains combined with ‘hem’ containing iron. The minor hemoglobin is A2, which consists of two alpha and two delta globin chains. Fetal hemoglobin (which declines after birth) is also present and consists of two alpha and two gamma globin chains. Several hundreds of different mutations or deletions of globin genes have been found, that may give rise to different forms of thalassemia. Alpha-thalassemia and beta-thalassemia are the most common and clinically relevant.

Beta-thalassemia results from different mutations of beta-globin genes leading to the absence of beta-globin chains (beta\textsuperscript{o} thalassemia) or their reduced synthesis (beta\textsuperscript{+} thalassemia), with an excess of alpha-globin chains. Severity is also variable from mild anemia, through intermediate forms, to severe anemia. In severe cases of beta-thalassemia (known as thalassemia major), other complications include bone deformation, enlarged spleen, and growth retardation.33

Thalassemia is found across the world. Affected population estimated rates are: Europe 0.9 percent, Asia 4.1 percent, Africa 13.3 percent, Oceana (including Australia, New Zealand, Papua New Guinea, Fiji) 1.3 percent, and America 2 percent.34 In many populations, where thalassemia is common, the genes for hemoglobin variants such as the sickle hemoglobin S, or hemoglobin C and E are also prevalent. Therefore, some people inherit a gene for beta-thalassemia from one parent and a gene for hemoglobin variant from the other. The most important diseases of this type are sickle beta-thalassemia and hemoglobin E beta-thalassemia. Over 4.5 percent of the global population carries a hemoglobin variant gene, however, almost three-quarters of affected births are in Africa.34

Phenotype classification of thalassemia is based on severity of anemia and need for transfusion therapy. Carriers with only one abnormal gene have thalassemia minor (mild anemia with minor hematological changes). People with two or more abnormal genes may inherit thalassemia intermedia (moderate anemia with typical body alterations (e.g. bone deformation, growth retardation) or thalassemia major (severe transfusion-dependent anemia). Life-long and regular blood transfusion is required to treat thalassemia major. This results in excessive accumulation of iron in the body (iron overload) that in the long term gives severe clinical complications such as heart and liver failure, diabetes, hypogonadism. Iron overload may be prevented and treated by daily removal (iron chelation therapy), which involves overnight infusions of desferrioxamine. Poor compliance with this treatment is the most common clinical problem in thalassemia major.35 Alternative chelating agents like oral deferiprone is also useful and effective,36 however, when used alone, it is inferior to desferrioxamine. Recently, combined therapy with desferrioxamine and deferiprone has been shown to be effective in refractory cases.37 Due to advances in transfusion regimens, chelation therapy, and the treatment of the complications of thalassemia, many patients can now lead active lives and have increased survival. Many of these patients are now treated as outpatients and in peripheral centers. Some patients are fortunate to receive bone marrow transplantation, which is limited to patients with a sibling of the same tissue type,38 and do well.

Treatment for thalassemia depends on the type and severity of the disease.

i. People who are carriers (thalassemia trait) usually have no symptoms and need no treatment.
ii. Those with moderate forms of thalassemia (for example, thalassemia intermedia) may need blood transfusions occasionally.
iii. Those with severe thalassemia have a serious and life-threatening illness. They are treated with regular blood transfusions, iron chelation therapy, and bone marrow transplants. Without treatment, children with severe thalassemia do not live beyond early childhood.

Adequate quantity and high quality of blood, transfused in the appropriate way, are the key concepts in the protocol for routine administration of blood to patients with thalassemia.39 The major goals are:

i. Maintenance of red cell viability and function during storage, to ensure sufficient transport of oxygen.
ii. Use of donor erythrocytes with a normal recovery and half-life in the recipient.

iii. Achievement of appropriate hemoglobin level.

iv. Avoidance of adverse reactions, including transmission of infectious agents.

Blood Transfusion Therapy

Blood transfusions should be given on a regular basis and not on a PRN basis generally every 2 to 5 weeks. It is preferable to transfuse in the day care center so that it does not disrupt the child’s school schedule or the family life. Every effort should be made not to transfuse before the diagnosis is confirmed by electrophoresis.39 Also, before the patient is transfused, the complete red cell genotype should be determined so that subsequent development of alloantibodies can be detected.

Donor Selection

Blood products for patients with thalassemia should be obtained from carefully selected healthy voluntary donors who have undergone extensive questioning and laboratory screening for hepatitis B, hepatitis C, HIV, and syphilis.39 Specific strategies for donor selection and product screening will be influenced by the prevalence of infectious agents in the donor population.

When to Start Blood Transfusions

When a diagnosis of homozygous thalassemia is made and the patient develops signs and symptoms of anemia with hemoglobin below 7 g/dl these patients should be put on regular transfusion program to offset the impaired growth and bony changes that are otherwise inevitable.39

Some patients with homozygous thalassemia do not drop their Hb but do so under stress-like infections (thalassemia intermedia). They may require episodic blood transfusions to tide over the stressful period, but does not require regular transfusion therapy.

What to Transfuse

Patients should be transfused with ABO and Rh compatible blood and should be monitored for the development of red cell alloantibodies. All attempts must be made to transfuse as fresh blood as possible (not older than 10–12 days) as 2,3-DPG gets depleted in stored blood reducing the capacity to deliver oxygen to the tissues.39 Decreased recovery and shortened half-life may increase transfusion requirements.

Recommended Blood Product

Patients with thalassemia should receive leukoreduced packed red cells.39 Reduction of leukocytes to $5 \times 10^6$ is considered the critical threshold for eliminating adverse reactions attributed to contaminating white cells and for preventing platelet alloimmunization.40 Methods for leukoreduction include:

i. Prestorage filtration of whole blood to remove white blood cells, carried out with an in-line filter within eight hours after blood collection. The delay in filtration may allow some phagocytosis of bacteria (e.g. *Yersinia enterocolitica*).41 This method of leukocyte removal offers high efficiency filtration and provides consistently low residual leukocytes in the processed red cells and high red cell recovery. Packed red cells are obtained by centrifugation of the leukoreduced whole blood.

ii. Bedside filtration: Red cells are obtained from stored whole blood by centrifugation to separate the plasma and remove the buffy coat. At the time of transfusion, the red cell unit is filtered at the bedside. This method may not allow optimal quality control because the techniques used for bedside filtration may be highly variable.

Blood Products for Special Patient Populations

Washed red cells may be beneficial for patients with thalassemia who have repeated severe allergic transfusion reactions.39 Saline washing removes plasma proteins in the donor product that are the target for antibodies in the recipient. Other clinical states that may require washed red cell products include immunoglobulin A (IgA) deficiency, in which the recipient’s preformed antibody to IgA may result in an anaphylactic reaction. Washing usually does not result in adequate leukocyte reduction and, therefore, should be used in conjunction with filtration. Washing of red cell units may remove some erythrocytes from the transfusion product.

Frozen red cells are used to maintain a supply of rare donor units for certain patients with unusual red
cell antibodies against missing common red cell antigens. The Euroblood Bank in Amsterdam, the Netherlands, provides a wide variety of special blood types.

Early clinical studies of neocytes, young red blood cells separated from older cells by density centrifugation, confirmed that a modest extension of transfusion interval could be achieved with their use. Later studies, which used a simpler method of preparation, confirmed these findings. However, it was observed that the reduction in total annual transfusional iron load during neocyte transfusions varied widely: from less than 10 percent to more than 25 percent. Cost-benefit analysis of neocyte transfusions remains complicated; the benefits of reduced iron administration are accompanied by an increased exposure to donated units and up to a fivefold increase in preparation expenses over those of standard concentrates.

Compatibility Testing

Development of one or more specific red cell antibodies (alloimmunization) is a common complication of chronic transfusion therapy. Thus, it is important to monitor patients carefully for the development of new antibodies and to eliminate donors with the corresponding antigens. Anti-E, anti-C and anti-Kell alloantibodies are most common. However, 5 to 10 percent of patients present with alloantibodies against rare erythrocyte antigens or with warm or cold antibodies of unidentified specificity.

Before embarking on transfusion therapy, patients should have extended red cell antigen typing that includes at least C, c, E, e, and Kell in order to help identify and characterize antibodies in case of later immunization. All patients with thalassemia should be transfused with ABO and Rh(D) compatible blood. Some clinicians recommend the use of blood that is also matched for at least the C, E and Kell antigens in order to avoid alloimmunization against these antigens. Some centers use even more extended antigen matching. However, it is advisable that:

1. Before each transfusion, one should perform a full cross-match and screen for new antibodies.
2. If new antibodies appear, they must be identified so that blood missing the corresponding antigen(s) can be used.
3. A complete record of antigen typing, red cell antibodies and transfusion reactions should be maintained for each patient, and should be readily available if the patient is transfused at a different center.
4. Transfusion of blood from first-degree relatives should be avoided because of the risk of developing antibodies that might adversely affect the outcome of a later bone marrow transplant.

Transfusion Regimens

Analysis of data of 10 years of administration of a ‘moderate’ transfusion regimen, in which mean pretransfusion hemoglobin concentrations did not exceed 9.5 g/dl, reported declines in transfusion requirements and in body iron loading, as estimated by serum ferritin concentration. Moreover, marrow activity did not increase more than threefold over normal, and there was a lower incidence of endocrine and cardiac complications.

The aim of transfusion therapy is thus to achieve a mean Hb concentration of 12 g/dl. This will suppress the patient’s production of defective red cells, secondary bony changes, hypersplenism, etc. The pretransfusion Hb should be around 9 to 10.5 g/dl and transfusion interval should be about 2 to 5 weeks, such that it will help achieve a mean Hb of 12 g/dl as planned. While shorter intervals between transfusions may reduce overall blood requirements, the choice of interval must take into account other factors like patients work or school schedule, how far he stays, how frequently he can come regularly, transport arrangements, and financial backing for all these endeavors.

This transfusion regimen promotes normal growth, allows normal physical activities, adequately suppresses bone marrow activity, and minimizes transfusional iron accumulation. Both ‘hypertransfusion’ and ‘supertransfusion’ regimens, in which pretransfusion hemoglobins are maintained above 10 and 12 g/dl, respectively, prevent most complications of anemia and ineffective erythropoiesis but are associated with substantial iron loading.

**How much Blood to Transfuse?**

The amount of blood to be transfused is calculated by the following formula:
• Packed red cell (ml) = 14-present Hb × weight (kg).
• Patients on monthly transfusions require 12 to 20 ml/kg of packed red cells. 3 ml/kg will increase Hb by 1 g/dl, but Hb falls by 1.5 g/dl/week (1 g/dl/week in splenectomized patients).
• One should not transfuse more than 2 units in 24 hours. The rate of infusion of blood should not be more than 6 ml/kg/hr (2–4 ml/kg/hr in patients with cardiac impairment). Give diuretics to avoid volume overload. Transfusion time should not exceed more than 4 hours to avoid bacterial contamination.

The recommended volume of transfused red cells is complicated by the use of different anticoagulant-preserved solutions. For CPD-A units with a hematocrit of approximately 75 percent, the volume per transfusion is usually 10 to 15 ml/kg, administered over 3 to 4 hours. Units with additive solutions may have hematocrits of 60 to 70 percent; and, therefore, larger volumes are needed to administer the same red cell mass as delivered by CPD-A units with a higher hematocrit. For most patients, it is usually easier to avoid these differences in red cell concentration by ordering a certain number of units (e.g. 1 or 2) rather than a particular volume of blood. Younger children may require a fraction of a unit to avoid under- or overtransfusion. Patients with cardiac failure or very low initial hemoglobin levels should receive smaller amounts of red cells at slower rates of infusion.

The post-transfusion Hb should not be greater than 15 g/dl. Regular measurement of the post-transfusion hemoglobin level is unnecessary. However, occasional determinations allow assessment of the rate of fall in the hemoglobin level between transfusions and may be useful in evaluating the effects of changes in the transfusion regimen, the degree of hypersplenism, or unexplained changes in response to transfusion.

Erythrocytapheresis, or automated red cell exchange, has been shown to reduce net blood requirements and the rate of transfusional iron loading. However, donor blood utilization increases by two- to threefold, increasing the cost and the risk of infection and alloimmunization.

A careful record of transfused blood should be maintained for each patient. This record should include the volume or weight of the administered units, the hematocrit of the units or the average hematocrit of units with similar anticoagulant-preserved solutions, and the patient’s weight. With this information, it is possible to calculate the annual blood requirements as ml of red cells per kg of body weight. A change in these requirements may be an important evidence of hypersplenism or accelerated destruction of donor red cells.

### Adverse Reactions

Blood transfusion exposes the patient to a variety of risks. Thus, it is vital to continue to improve blood safety and to find ways of reducing transfusion requirements and the number of donor exposures. Adverse events associated with transfusion include:

i. **Nonhemolytic febrile transfusion reactions**: These were common in the past decades, but have been dramatically reduced by leukoreduction. If effective leukoreduction is not possible, patients experiencing these reactions should be given antipyretics before their transfusions.

ii. **Allergic reactions**: Ranging from mild to severe, these are mainly due to plasma proteins. They have been markedly reduced by plasma removal. A patient prone to allergic reactions may benefit from washed red cells.

iii. **Acute hemolytic reactions**: These are unusual, and most commonly arise from errors in patient identification or blood typing and compatibility testing. The risk of receiving the wrong blood is greater for a thalassemic patient who travels to another center or is admitted to a hospital not familiar with him/her. Hemolytic reactions in these patients can still be avoided if the blood bank is familiar with the World Health Organization (WHO) protocol for screening for antibodies and carrying out the necessary full crossmatching of donor units.

iv. **Autoimmune hemolytic anemia**: It is a very serious complication of transfusion therapy and is usually combined with underlying alloimmunization. Even red cells from seemingly compatible units may have markedly shortened survival, and the hemoglobin concentration may fall well below the usual pretransfusion level. Destruction of both the donor red cells and the recipient’s red cells occurs. Steroids, immunosuppressive drugs and intravenous immunoglobulin are used for the clinical management of this situation, although
they may give little benefit. This complication may occur more frequently in patients who begin transfusion therapy later in life.

v. Delayed transfusion reactions: These occur 5 to 10 days after transfusion and are characterized by anemia, malaise, and jaundice. These reactions may be due to an alloantibody that was not detectable at the time of transfusion or to the development of a new antibody. A sample should be sent to the blood bank to look for a new antibody and to re-crossmatch the last administered units.

vi. Transfusion-related acute lung injury (TRALI) and graft versus host disease (GVHD), rare but clinically very severe conditions. TRALI, caused by specific antineutrophil or anti-HLA antibodies, is characterized by dyspnea, tachycardia, fever and hypotension. Management includes oxygen, administration of steroids and diuretics, and, when needed, assisted ventilation. GVHD is a particular hazard when the donor is a family member who shares HLA haplotypes with an immunosuppressed recipient. Donated blood from a family member should be irradiated before transfusion.

vii. Transmission of infectious agents including viruses, bacteria and parasites, a major risk in blood transfusion. New problems continue to materialize, such as the discovery of new viruses (e.g. HGV, TTV, SEN-V) as well as the re-emergence of old, forgotten infectious agents, like the new variant of Creutzfeldt-Jakob Disease. Continued transmission of hepatitis B, hepatitis C and HIV underscore the importance of voluntary blood donations, careful donor screening, and, in the case of hepatitis B, immunization.

**Hepatitis C Infection**

Iron overload and infection with hepatitis C virus act as co-factors in the evolution of chronic liver disease, a common cause of death after age 15 years in thalassemia major. From 60 to 80 percent of transfused thalassemic adults have evidence of previous infection with hepatitis C virus, and a substantial proportion of these progress to cirrhosis, liver failure and hepatocellular carcinoma. Recombinant alpha-interferon is the only effective therapy for chronic infection with hepatitis C virus; alpha-interferon leads to sustained biochemical and histological responses in about 25 percent of infected patients. A recent study of long-term efficacy of alpha-interferon in patients with thalassemia major recently reported a complete sustained response, defined as both sustained normalization of serum alanine aminotransferase (ALT) and clearance of hepatitis C virus RNA from serum, in 40 percent of patients over a mean follow-up period of 3 years. The rate of relapse was much lower in thalassemic (18%) than in nonthalassemic (50%) individuals, but a more prolonged period of treatment was required in thalassemia patients. Practically, this suggests that alpha-interferon in most patients with thalassemia not be stopped after 3 to 6 months, but should be continued at least until serum ALT declines to normal. This and previous studies have highlighted the importance of compliance with desferoxamine during alpha-interferon therapy. Other strategies to improve response in thalassemia to alpha-interferon, including its co-administration with ribavirin are under evaluation.

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Hemorrhagic Disorders in the Surgical Patient

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Bleeding is a potential complication of almost every surgical procedure; and when appropriately anticipated, it can be managed with relative ease. This chapter reviews aspects of the pathophysiology of bleeding that are important to the surgeon. By way of definition, the term “hemostasis” is used in reference to the normal physiologic (and usually desirable) blood clotting response to breaching of blood vessel integrity. Hemorrhage is a term that refers to the pathologic (and therefore undesirable) heavy or uncontrollable bleeding. Hemostasis is a complex interaction of platelets, coagulation factors, blood vessel walls and the inhibitory pathways. Potential perturbations of any of these elements, either individually or collectively, may give rise to excessive bleeding in the postoperative period. These perturbations can either be hereditary or acquired. Preoperative detection of these defects is the key to minimizing potential complications. Timely recognition and management of hemorrhage is fundamental to good surgical care.

PHYSIOLOGY OF HEMOSTASIS

Platelets, coagulation proteins and the blood vessel wall act in concert to achieve hemostasis. Disruption of the blood vessel wall, either by trauma or by incision exposes the vascular subintimal tissue and activates a sequence of catalytic events that culminate in clot formation. A severe abnormality of one component or a modest abnormality of two or more of these components may result in a hemostatic disorder postoperatively.

Role of Platelets

Primary hemostasis is initiated when circulating platelets are exposed to the vascular subendothelium, and adhere specifically to it and its rich content of procoagulant proteins, such as von Willebrand’s factor (vWF), collagen and fibronectin. Once adherent, platelets undergo an energy-dependent shape change and release preformed substances that serve several functions:
1. Vasoconstriction, mediated by thromboxane A₂, epinephrine and serotonin, assists in the control of local hemorrhage.
2. Exposure of phosphatidyl serine on the platelet surface provides a template for local assembly of coagulation protein enzymatic complexes for further hemostasis (see below).
3. Initiation of blood vessel wall repair by platelet-derived growth factor and angiogenic factors. More platelets are recruited to the site of injury, and they aggregate to form a platelet plug, which is then stabilized by the insoluble fibrin meshwork. For details, see reference.¹

Role of the Coagulation Proteins

Secondary hemostasis: The principal function of the coagulation pathway is to form a hemostatic plug, whereby soluble fibrinogen is converted to insoluble fibrin at the site of the injured vessel wall. Earlier, “cascade” or “waterfall” theories of coagulation separated the coagulation pathway into “intrinsic” (contact pathway) and “extrinsic” (tissue factor pathway) systems. However, in the last couple of
decades, it has become increasingly clear that *in vivo*,
there is probably only one pathway by which coagula-
tion is initiated, namely the tissue factor pathway. It
is now believed that coagulation is initiated by the
formation of a complex between tissue factor (TF), a
protein which is expressed on certain cell surfaces (see
below), and circulating activated factor VII (fVIIa) (Fig.
16.1).

In order to preserve the essential hemostatic
function of TF, while avoiding unwanted thrombosis,
it is generally believed that TF is not normally exposed
to the circulating blood, but is found in cells located
in the outermost layers of vessel walls. Following
injury to a vessel wall, TF is exposed to the circulating
blood, and forms complexes with fVIIa. About 1
percent of the total factor VII protein normally is
present in circulation in the activated form (i.e. fVIIa).
The TF-fVIIa complex activates factor X (fX), which
then converts prothrombin (fII) to thrombin (fIIa).
Thrombin then performs an amplification function by
activating factors VIII and V, as well as platelets
accumulated at the site of injury. This is often referred
to as the propagation phase of coagulation. These
activated platelets expose phosphatidyl serine on their
surfaces, thereby forming a template for further hemostasis.

The TF-fVIIa complex also activates factor IX (fIX)
when it is bound to phosphatidyl serine on activated
platelets. A complex is formed between fIXa and its
cofactor, fVIIIa, which in turn activates fX to fXa. FXa
complexes with fVa and converts additional fII to fIIa.
All these processes occur on activated platelet surfaces
and result in the generation of high concentrations of
thrombin (“full thrombin burst”), necessary for
formation of a solid fibrin hemostatic plug. Thrombin
also activates (a) factor XIII, which acts as a fibrin
stabilizing factor; and (b) thrombin activated
fibrinolysis inhibitor (TAFI), which serves to render
the fibrin plug more resistant to plasmin.

There are numerous mechanisms in place to limit
the degree of clot progression. These include:
1. Tissue factor pathway inhibitor (TFPI), which
binds to fXa and then inhibits the TF-fVIIa complex.
2. Activated protein C (APC), which complexes with
its cofactor, protein S, on the surface of platelets
and endothelial cells to proteolytically inactivate

![Cell-based model of coagulation cascade](image)

**Fig. 16.1:** The coagulation pathway is triggered by the TF/fVIIa complex, leading to the generation of sufficient amount of thrombin
to activate platelets, factors V, VIII, and XI, before it is shut down by TFPI. The fVIIa/fIXa complex then activates fX on the
activated platelet surface, leading to a further “thrombin burst”
fVa and fVIIIa, thus destroying their coagulant activity. Protein C in turn is activated by the thrombin-thrombomodulin complex (thrombomodulin is expressed on endothelial cells).

3. Antithrombin (III), which rapidly blocks free thrombin, and also factors IXa, Xa, XIa, and XIIa. Its inhibitory activity is greatly accelerated by the presence of exogenous heparin or endogenous heparin-like molecules.

4. Heparin cofactor II, which acts as a secondary inhibitor of thrombin. This effect is enhanced in the presence of endothelial dermatan sulfate and heparin.

5. Tissue plasminogen activator (t-PA) released from the endothelial cell, which converts plasminogen to plasmin. Plasmin is responsible for fibrinolysis, thus reducing the size of the clot and initiating events that lead to vascular repair.

Please see references2,3 for more details on cell-based model of hemostasis.

Role of Vascular Endothelium

The endothelium is more than just an inert lining layer on the inner surface of blood vessels. Indeed, various substances synthesized by the vascular endothelium play an important role in hemostasis, as summarized in Table 16.1.

BLEEDING DISORDERS

Identification of a bleeding diathesis may indicate a significant risk of intraoperative bleeding and its associated complications (including wound infection, joint and muscle hematomas, and pressure necrosis). A working knowledge of the major components of hemostasis helps identify potential defects, including:

1. Defects in the platelet plug formation, either due to platelet or vessel wall defects.
2. Defects in the formation of a stable fibrin clot.
3. Excessive degradation or fibrinolysis of the thrombus.

The best screening test for a patient’s bleeding risk preoperatively is a careful bleeding history, including a history of bleeding with trauma, past surgical procedures (including tonsillectomy and circumcision, if performed), and dental procedures. A propensity for excessive mucocutaneous bleeding is suggestive of a primary hemostatic disorder, including platelet deficiency or dysfunction, vascular fragility disorders, and von Willebrand’s disease (vWD). A predilection for soft tissue/joint bleeding is more suggestive of coagulation protein deficiencies, including hemophilia A and B. Delayed bleeding after surgical procedures may be indicative of Factor XIII deficiency. A medication list should also be sought, as a number of agents can inhibit platelet function (Table 16.2).

Although the likelihood of provoking serious bleeding with the use of aspirin and other NSAIDs by themselves is relatively low, their use may magnify other hemostatic defects; for example, von Willebrand’s disease, mild hemophilia, uremia or liver disease. A detailed family history for bleeding propensity and its inheritance pattern may raise the suspicion for some of the hereditary forms of bleeding disorders.

Screening laboratory tests have an important role in the preoperative evaluation in combination with a good history and physical examination. However, given the variety of potential hemostatic defects, there is no single simple screening laboratory test; and in general, routine assays offer a limited predictive value for perioperative bleeding. In patients undergoing surgeries with a low risk of bleeding (including surgery of non-vital organs; exposed surgical site; limited dissection), no additional laboratory tests are required if the history is not suggestive of a bleeding tendency. For patients undergoing surgeries with moderate-to-high risk of bleeding (including surgery involving vital organs; deep or extensive dissection; bleeding likely to compromise surgical result; bleeding complications frequent), screening tests may include platelet count, platelet function assessment by measuring collagen/epinephrine closure time using whole blood Platelet Function Analyzer device (PFA-

### Table 16.1: Endothelial cell regulation of coagulation

<table>
<thead>
<tr>
<th>Factor</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Willebrand’s factor</td>
<td>Promotes platelet adhesion</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>Binds thrombin and activates protein C</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Inhibits fibrinolysis</td>
</tr>
<tr>
<td>t-PA</td>
<td>Promotes fibrinolysis</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Initiates coagulation</td>
</tr>
<tr>
<td>PGI2 (prostacyclin)</td>
<td>Impairs platelet aggregation / vasodilator</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Vasodilator</td>
</tr>
<tr>
<td>Endothelins (ET 1, 2, 3)</td>
<td>Vasoconstrictors</td>
</tr>
</tbody>
</table>
Hemorrhagic Disorders in the Surgical Patient

100®, Dade-Behring), PTT and INR, irrespective of bleeding history (Table 16.4). There is a move away from performing the bleeding time routinely, as it is very insensitive in predicting a patient’s bleeding risk, and is dependent on numerous variables. The PFA-100® device seems to offer more information on primary hemostasis (i.e. is more sensitive and specific) than the bleeding time.

Thrombocytopenia or platelet dysfunction can lead to abnormal closure times measured by the whole-blood platelet function analyzer. A prolonged collagen/epinephrine (Col/Epi) closure time may also be due to aspirin ingestion leading to platelet dysfunction. If one reading is abnormal, it should be repeated approximately 7 days after discontinuation of aspirin, along with the collagen/ADP (Col/ADP) closure time. If one or either closure times remain prolonged, it is suggestive of an intrinsic platelet dysfunction. In patients with von Willebrand’s disease, both Col/Epi and Col/ADP closure times are typically prolonged. For additional reading, please see references.4,5 An approach to further work up of an abnormal coagulation profile is outlined in Figure 16.2.

Preoperative and Postoperative Management of a Patient with Hemostatic Defect

Identifying the type and severity of the hemostatic defect resulting in the hemorrhagic diathesis will direct the therapy. For example, if the defect is determined to be a specific coagulation factor deficiency, it can usually be corrected by replacing the deficient factor, either with specific factor concentrate, fresh frozen plasma or with cryoprecipitate to restore a level sufficient to achieve normal hemostasis (Table 16.5). For low-risk surgeries, patients with mild hemophilia A (fVIII deficiency) and von Willebrand’s disease (types 1 and 2a) may benefit from preoperative use of deamino-8-D-arginine vasopressin (DDAVP), which promotes release of preformed von Willebrand factor and fVIII from vascular endothelial cells. In addition, antifibrinolytic agents such as aminocaproic acid may be added postoperatively. DDAVP may also be used in patients with uremic platelet dysfunction, where it has been shown to improve hemostasis.

Platelet functional defects (including Glanzmann’s thrombasthenia, gray platelet syndrome, storage pool deficiency, Wiskott-Aldrich syndrome, Bernard-Soulier syndrome) are managed with transfusion of

---

Table 16.2: Drugs inhibiting platelet function

<table>
<thead>
<tr>
<th>Nonsteroidal anti-inflammatory drugs</th>
<th>Glycoprotein IIb/IIIa inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Abciximab</td>
</tr>
<tr>
<td>Sulfinpyrazone</td>
<td>Epifibatide</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Tiroliban</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Lamifibean</td>
</tr>
<tr>
<td>Sulindac</td>
<td>Psychotropic drugs</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Tricyclic antidepressants</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Imipramine</td>
</tr>
<tr>
<td>Meclofenamic acid</td>
<td>Amtriptyline</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>Nortriptyline</td>
</tr>
<tr>
<td>Diflunisal</td>
<td>Pheno thiazines</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>Chlorpromazine</td>
</tr>
<tr>
<td>Tolmetin</td>
<td>Trifluoperazine</td>
</tr>
</tbody>
</table>

Beta-lactam antibiotics

Penicillins

| Penicillin G                      | Local                            |
| Carbenicillin                     | Lidocaine                        |
| Ticarcillin                       | Tetracaine                       |
| Methicillin                       | Butacaine                        |
| Ampicillin                        | Cyclaine                         |
| Nafcillin                         | Nupercaine                       |
| Piperacillin                      | Procaine                         |
| Azlocillin                        | Cocaine                          |
| Mezlocillin                       | Plaquenil                        |
| Cephalosporins                    | General                           |
| Cephalothin                       | Halothane                        |

Moxalactam

Cefoxitin

Cefotaxime

Cefazolin

Other drugs

Antibiotics

Nitrofurantoin

Drugs that increase platelet cAMP

Prostacyclin

Iloprost

Dipyrimidine

Anticoagulants

Heparin

Plasminogen activators

Plasma expanders

Dextran

Hydroxyethyl starch (Hextend)

Cardiovascular drugs

Nitroglycerine

Isosorbide dinitrate

Propranolol

Nitroprusside

Nifedipine

Verapamil

Diltiazem

Quinidine

<table>
<thead>
<tr>
<th>Drugs inhibiting platelet function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsteroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Sulfinpyrazone</td>
</tr>
<tr>
<td>Indomethacin</td>
</tr>
<tr>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Sulindac</td>
</tr>
<tr>
<td>Naproxen</td>
</tr>
<tr>
<td>Phenylbutazone</td>
</tr>
<tr>
<td>Meclofenamic acid</td>
</tr>
<tr>
<td>Mefenamic acid</td>
</tr>
<tr>
<td>Diflunisal</td>
</tr>
<tr>
<td>Piroxicam</td>
</tr>
<tr>
<td>Tolmetin</td>
</tr>
<tr>
<td>Cephalosporins</td>
</tr>
<tr>
<td>Cephalothin</td>
</tr>
<tr>
<td>Moxalactam</td>
</tr>
<tr>
<td>Cefoxitin</td>
</tr>
<tr>
<td>Cefotaxime</td>
</tr>
<tr>
<td>Cefazolin</td>
</tr>
<tr>
<td>Antibiotics</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
</tr>
<tr>
<td>Drugs that increase platelet cAMP</td>
</tr>
<tr>
<td>Prostacyclin</td>
</tr>
<tr>
<td>Iloprost</td>
</tr>
<tr>
<td>Dipyrimidine</td>
</tr>
<tr>
<td>Anticoagulants</td>
</tr>
<tr>
<td>Heparin</td>
</tr>
<tr>
<td>Plasminogen activators</td>
</tr>
<tr>
<td>Plasma expanders</td>
</tr>
<tr>
<td>Dextran</td>
</tr>
<tr>
<td>Hydroxyethyl starch (Hextend)</td>
</tr>
<tr>
<td>Cardiovascular drugs</td>
</tr>
<tr>
<td>Nitroglycerine</td>
</tr>
<tr>
<td>Isosorbide dinitrate</td>
</tr>
<tr>
<td>Propranolol</td>
</tr>
<tr>
<td>Nitroprusside</td>
</tr>
<tr>
<td>Nifedipine</td>
</tr>
<tr>
<td>Verapamil</td>
</tr>
<tr>
<td>Diltiazem</td>
</tr>
<tr>
<td>Quinidine</td>
</tr>
</tbody>
</table>

**Fig. 16.2:** Approach to evaluation of the screening coagulation profile in the patient with an abnormal bleeding history


### Table 16.3: Some hereditary bleeding disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Screening laboratory abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A (Factor VIII deficiency)</td>
<td>Prolonged PTT</td>
</tr>
<tr>
<td>Hemophilia B (Factor IX deficiency)</td>
<td>Prolonged PTT</td>
</tr>
<tr>
<td>von Willebrand’s disease</td>
<td>Abnormal PFA-100 closure time; prolonged bleeding time; +/- prolonged PTT</td>
</tr>
<tr>
<td>Hereditary hemorrhagic telangiectasia (Osler-Rendu-Weber syndrome)</td>
<td>None</td>
</tr>
<tr>
<td>Glanzmann’s thrombasthenia</td>
<td>Abnormal PFA-100 closure time; prolonged bleeding time</td>
</tr>
<tr>
<td>Bernard-Soulier syndrome</td>
<td>Abnormal PFA-100 closure time; prolonged bleeding time; low platelet count</td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>Abnormal PFA-100 closure time; prolonged bleeding time</td>
</tr>
</tbody>
</table>

platelets. Transfusion of 5 units of platelets preoperatively, with availability of an additional 5 units during the operative procedure is usually adequate. Thrombocytopenia due to inadequate megakaryopoiesis may also be treated with platelet transfusion, but it is of limited value alone for the management of destructive (idiopathic thrombocytopenic purpura) or sequestrative thrombo-
Excessive bleeding in the intraoperative and postoperative periods can be potentially life threatening, and requires prompt evaluation and institution of therapy. It is vital to evaluate whether the bleeding is due to hemostatic failure or secondary to a local cause that may be surgically correctable. Failure to achieve adequate hemostasis at the operative site is the most frequent cause of postoperative bleeding. Excessive, rapid blood loss limited to the surgical site may be due to bleeding from a large vessel. In contrast, bleeding due to a hemostatic defect is typically a more widespread “oozing” at the operative site, which may also involve additional sites outside the surgical field. Therefore, patients should be examined for other bleeding sites, including petechiae, ecchymosis, gastrointestinal or genitourinary bleed, and bleeding from venipuncture and/or central venous catheter sites.

An approach similar to that recommended for preoperative screening should be utilized in the post-operative evaluation and management of a hemorrhagic diathesis. A pre-existing hemostatic defect may not have been detected preoperatively; therefore, the patients past medical and family history should be reviewed. The list of medications should be reviewed again, and if possible, medications that...

---

**Table 16.4: Preoperative screening for bleeding disorders**

<table>
<thead>
<tr>
<th>Bleeding risk</th>
<th>Bleeding history</th>
<th>Surgical procedure</th>
<th>Laboratory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal</td>
<td>Negative</td>
<td>Low risk/minor (e.g. dental extraction, lymph node biopsy)</td>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
<td>Negative</td>
<td>Moderate risk/major (e.g. cholecystectomy, laparotomy, bowel resection)</td>
<td>INR, PTT, platelet count</td>
</tr>
<tr>
<td>High</td>
<td>Questionable or negative</td>
<td>High risk (e.g. CNS surgery, cardiopulmonary bypass surgeries, prostatectomy)</td>
<td>INR, PTT, platelet count, PFA-100® Col/Epi closure time</td>
</tr>
<tr>
<td>Very high</td>
<td>Positive</td>
<td>Minor or major</td>
<td>Same as “High” group. If screen normal, consider platelet aggregation studies, factor VIII and IX assays, TT, and screening for conditions mentioned in last column of Figure 16.2 (bleeding patients normal INR and PTT)</td>
</tr>
</tbody>
</table>

**Table 16.5: Plasma clotting factors and source of replacement**

<table>
<thead>
<tr>
<th>Factor</th>
<th>In vivo half-life</th>
<th>Minimum plasma level required for hemostasis</th>
<th>Source of factor replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>3-4 days</td>
<td>100 mg/dl</td>
<td>Cryoprecipitate</td>
</tr>
<tr>
<td>II</td>
<td>2-5 days</td>
<td>20-40 %</td>
<td>FFP²</td>
</tr>
<tr>
<td>V</td>
<td>15-36 hours</td>
<td>25-30%</td>
<td>FFP</td>
</tr>
<tr>
<td>VII</td>
<td>4-7 hours</td>
<td>10-20%</td>
<td>FFP or rf VIIa (NovoSeven)</td>
</tr>
<tr>
<td>VIII</td>
<td>8-12 hours</td>
<td>25-30%</td>
<td>Recombinant or plasma derived factor VIII concentrate</td>
</tr>
<tr>
<td>IX</td>
<td>20-24 hours</td>
<td>25-30%</td>
<td>Recombinant or plasma derived factor IX concentrate</td>
</tr>
<tr>
<td>X</td>
<td>32-48 hours</td>
<td>10-20%</td>
<td>FFP</td>
</tr>
<tr>
<td>XI</td>
<td>3 days</td>
<td>15-25%</td>
<td>FFP</td>
</tr>
<tr>
<td>XII</td>
<td>48-52 hours</td>
<td>0%</td>
<td>(correction not required)</td>
</tr>
<tr>
<td>XIII</td>
<td>12 days</td>
<td>&lt;5%</td>
<td>FFP (or cryoprecipitate)</td>
</tr>
<tr>
<td>von Willebrand’s</td>
<td>8-12 hours</td>
<td>25-50%</td>
<td>Intermediate purity, plasma-derived concentrate (Humate-P, Alphanate)</td>
</tr>
</tbody>
</table>

¹ Percentage values expressed as percentage of normal  
² FFP=fresh frozen plasma
can potentially interfere with the coagulation system should be discontinued. It should be remembered that many over-the-counter medications, including cold remedies, include aspirin. Furthermore, patients are at risk of developing vitamin K deficiency postoperatively (especially if oral intake has been poor and broad-spectrum antibiotics have been employed). The bleeding diathesis in this case is due to an acquired deficiency of vitamin K-dependent factors (factors II, VII, IX and X). In addition to INR, PTT, thrombin time (TT), platelet count, and Col/Epi closure time, a peripheral blood smear, fibrinogen, and D-Dimer (or fibrin degradation products) should be examined to rule out disseminated intravascular coagulation (DIC). Factors VIII, IX and XI may be checked to rule out mild congenital factor deficiencies that may not prolong the PTT, especially if there is a family history of hemorrhagic diathesis. Many patients in the postoperative phase receive heparin, either via indwelling venous catheter flushes, or as antithrombotic prophylactic or therapeutic strategies, thus complicating the laboratory evaluation of the coagulation system. A normal reptilase time, in conjunction with prolonged PTT and TT is suggestive of heparin effect. Euglobulin clot lysis time, α2-antiplasmin, and plasminogen activator inhibitor-1 levels may be checked if a defect in the fibrinolytic system is suspected. Massive blood transfusion (i.e. transfusion of more than one blood volume ~5,000 ml) may lead to dilutional thrombocytopenia and coagulation factor deficiency and an excessive buildup of citrate that along with other hemostatic abnormalities may lead to bleeding.

Therapy is dependent on the hemostatic defect identified, as discussed earlier in the section on preoperative evaluation.

SPECIAL HEMOSTATIC CHALLENGES

Certain medical and surgical conditions pose special hemostatic challenges. These include:
1. cardiopulmonary bypass surgery;
2. liver disease;
3. renal disease.

Cardiopulmonary Bypass Surgery

Postoperative bleeding incidence following cardiopulmonary bypass (CPB) surgery is 0.8 to 5 percent, and may be profound, requiring reoperation. Perfusion of blood through the extracorporeal membrane oxygenator leads to hemostatic changes during CPB. The hemoglobin, platelet count, and levels of coagulation and fibrinolytic factors are reduced to ~50 percent (factor V level may be <20%) of baseline. This may be due to: (a) exposure to artificial surfaces; (b) tissue factor-dependent coagulation related to the surgical trauma with resultant depletion of the factors. Furthermore, the bypass procedure leads to significant platelet dysfunction due to release of granules, and generation of platelet micro-particles. The result may be abnormal in vitro platelet aggregation tests, and a prolonged bleeding time that typically corrects within 1 hour postoperatively. Other factors, such as inadequate neutralization of heparin by protamine sulfate also contribute to the bleeding disorder.

Management of the bleeding complications after CPB should include additional dose(s) of protamine sulfate, if heparin has been inadequately reversed, and supportive care with use of cryoprecipitate, fresh frozen plasma and vitamin K for patients with acquired coagulation factor deficiencies. Studies have not demonstrated a beneficial effect of platelet transfusion. The administration of DDAVP may enhance coagulation and decrease overall blood loss but concerns regarding its potential to increase the rate of postoperative myocardial infarction have been raised. Antifibrinolytic agents such as aprotinin, epsilon-aminocaproic acid and tranexamic acid have been shown to decrease both bleeding and transfusion requirements, without apparently increasing the postoperative thrombosis risk. For additional reading, please see references.6,7

Liver Disease

The liver plays a key role in coagulation. Bleeding complications in patients with acute or chronic liver disease may occur due to the following causes:
1. Factor deficiencies, either due to decreased production, vitamin K deficiency, or synthesis of abnormal coagulation factors. All coagulation factors with the exception of vWF and fVIII are synthesized by hepatocytes. Prolongation of the prothrombin time (PT/INR) is an indicator of the severity of liver disease, and is used as a prognostic
marker (in the Child-Pugh and Mayo indices of end-stage liver disease). A concomitant deficiency of vitamin K leads to further reduction of factors II, VII, IX, X, and proteins C and S, since vitamin K is required as a cofactor for hepatic $\gamma$-carboxylation of glutamic acid residues in the amino-terminal region of these proteins. The $\gamma$-carboxylated residues allow calcium ion binding, which is essential for the coagulation factor binding to phospholipids membranes and thus their functional activity.

2. **Dysfibrinogenemia** is the commonest qualitative factor defect in severe liver disease. An excessive number of sialic acid residues on fibrinogen interferes with enzymatic activity of thrombin, and is characterized by abnormal polymerization of fibrin monomers, leading to a prolonged thrombin time, and a reduced (functional) level of fibrinogen.

3. **Qualitative and quantitative platelet defects.** Thrombocytopenia is a common feature in patients with chronic liver disease, but is rarely below 30 to 40,000/mm$^3$. Splenomegaly due to portal hypertension is considered to be the main cause of low platelet count in cirrhosis. Additionally, thrombopoietin (TPO) is a cytokine produced by the liver, which is responsible for the maturation of megakaryocytes and the production of platelets. Serum levels of TPO are reduced in patients with liver disease and thrombocytopenia. Furthermore, the platelet count may be reduced secondary to disseminated intravascular coagulation (see below). Alcohol abuse, a major cause of chronic liver disease, can result in thrombocytopenia by either directly inhibiting bone marrow synthesis or by associated nutritional deficiency of folic acid. Thrombocytopenia in patients with hepatitis C virus, which is another common cause of chronic liver disease, may be due to immune-mediated platelet destruction, or from direct bone marrow suppression from the therapies used to manage it.

4. **Hyperfibrinolysis.** High circulating levels of tissue plasminogen activator (tPA), due to decreased hepatic clearance accounts for hyperfibrinolysis in end-stage liver disease. Moreover, progressive liver disease leads to low levels of $\alpha_2$-antiplasmin and thrombin activable fibrinolysis inhibitor (TAFI), thus further accelerating fibrinolysis.

5. **Accelerated intravascular coagulation.** This phenomenon is seen in patients with advanced liver disease, and the coagulation abnormalities are similar to low-grade disseminated intravascular coagulation (see below), although the widespread deposition of intravascular fibrin in the small vessels (typical of DIC) is usually absent in end-stage liver disease.

Spontaneous bleeding is infrequent in patients with advanced liver disease, and often no therapy is required for the coagulation defect. However, correction of the coagulation defect should be pursued if the patient has active bleeding, or is undergoing an invasive procedure or surgery. Administration of 10 to 20 ml/kg body weight of fresh frozen plasma usually shortens PT/INR promptly, but lasts no more than 12 to 24 hours. Vitamin K deficiency is likely in a patient with cholestatic liver disease; thus, a trial of 10 mg of vitamin K a day for 3 days (given orally or by slow intravenous administration) is a reasonable strategy to attempt normalization of the prolonged PT/INR. Subcutaneous vitamin K has an inconsistent rate of absorption, and has been demonstrated to be less efficacious than the oral or intravenous route of administration. Cryoprecipitate may be administered in a bleeding patient when the fibrinogen level is less than 100 mg/dl. One unit of administered cryoprecipitate for every 10 kg body weight typically increases plasma fibrinogen by 50 mg/dl. Recombinant factor VIIa is a promising although expensive agent for the correction of the hemostatic defect in patients with liver failure, and it may reduce blood product requirements during liver transplantation. However, further study is needed before its routine use can be endorsed. Platelet transfusions are required in patients with persistent bleeding if the platelet count is less than 50,000/mm$^3$, and an attempt to maintain the count above 100,000/mm$^3$ should be made. Prophylactic platelet transfusion is indicated only for patients undergoing invasive procedures or elective surgeries when the platelet count is less than 60,000/mm$^3$. DDAVP has not been shown to benefit patients with bleeding esophageal varices. Antifibrinolytic agents such as aprotinin, epsilon-aminocaproic acid, and tranexamic acid have demonstrated to be beneficial in controlling blood loss during orthotopic liver transplantation. However, care should be
exercised in the use of these agents in patients with DIC, as they may increase the risk of thrombosis. For additional reading, please see reference.8

Renal Disease

Chronic renal insufficiency affects the blood cell production, the complement and hemostatic systems, which include anemia, qualitative and quantitative platelet defects, coagulation factor deficiency, and blood vessel wall defects. These changes enhance the risk of bleeding. However, it should be noted that since the advent and routine use of recombinant erythropoietin in patients with chronic renal insufficiency, bleeding complications have declined dramatically.

Changes in the hemostatic system in renal insufficiency can make an individual more prone to either hemorrhage or thrombosis. The retention of low molecular weight toxins (e.g. guanidinosuccinic acid, phenolic acid) decrease adenosine diphosphate (ADP)-induced platelet aggregation, thereby predisposing to hemorrhage. By contrast, the vasodilatory prostaglandins activate platelets and induce activation of the coagulation cascade. In patients undergoing hemodialysis, the dialyzer membrane composition and flow design may also play a role in activating platelets. Decreased levels of factors IX, XI, and XII have been reported, probably explained by their relatively low molecular weight, which facilitates glomerular filtration. In contrast, factors II, V, VII, VIII, X and XIII are often increased; however, no relationship has been established between these increased factor levels and renal vein thrombosis.

A qualitative defect of von Willebrand’s factor has also been noted in uremia. In patients with bleeding complications or those undergoing surgery, intravenous infusion of 0.3 mcg/kg DDAVP may be tried, as it releases preformed vWF from the α-granules of platelets. Dosing may be repeated every 24 to 48 hours. More frequent administration of DDAVP is not effective due to tachyphylaxis, and it may lead to water retention with hyponatremia. Therefore, the patient’s fluid intake should be restricted to 1 liter/24 hours after the administration of DDAVP. If this is not effective in controlling bleeding, cryoprecipitate may be considered, realizing that there is a risk of transmission of viral infections. Alternatively, intermediate purity factor VIII products (e.g. Humate-P, Alphanate) that have high levels of vWF may be tried, although adequate data is not available to support their routine use. Other potential therapeutic modalities include the use of conjugated estrogens, but the onset of their peak hemostatic effect is delayed.

DISSEMINATED INTRAVASCULAR COAGULATION

By consensus, disseminated intravascular coagulation (DIC) is defined as “an acquired syndrome characterized by the intravascular activation of coagulation

| Table 16.6: Treatment options for common hemostatic problems prior to surgery |
|---------------------------------|-----------------|-----------------|
| Thrombocytopenia                | ≥ 80,000/mm³    | Bleeding unlikely |
| Platelet count                  | <80,000 and >20,000/mm³ | Platelet transfusion may be needed |
| < 20,000/mm³                    |                 | Bleeding likely, platelet transfusion indicated |
| Acquired platelet dysfunction   |                 |                 |
| (bleeding history)              |                 |                 |
| PFA Col/Epi closure time        | >180 sec        | DDAVP            |
| Bleeding time                   | >15 min         | Platelet transfusion if DDAVP ineffective |
| Liver disease                   |                 |                 |
| Prothrombin time                | ≤ 3 sec over control | Bleeding unlikely |
| Fibrinogen                      | > 3 sec over control | FFP indicated, vitamin K may be helpful |
| Thrombocytopenia                | < 100 mg/dl     | Cryoprecipitate indicated |
| Accelerated fibrinolysis        |                 | As above         |

Hemorrhagic Disorders in the Surgical Patient

with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction. The clinical manifestation can vary, and range from laboratory abnormalities alone to hemorrhagic and/or thrombotic complications. Bleeding is the predominant clinical manifestation of DIC, and is reported in 70 to 90 percent of patients. Commonly reported sites of bleeding include the skin, lungs, gastrointestinal and genitourinary tracts, and bleeding at surgical and vascular access sites. Thromboembolic manifestations are less frequent (10–40%), and are seen more often in patients with underlying malignancies or with pneumococcal or meningococcal sepsis.

Numerous disorders are associated with the development of DIC, and these are listed in Table 16.7. Regardless of the underlying cause, the final common pathway in the pathogenesis of DIC is an unregulated and excessive generation of thrombin due to the failure of the mechanisms that regulate thrombin generation (please refer to the section “Physiology of Hemostasis” for additional details; also see Table 16.8). The coagulation cascade is triggered by the exposure of blood to excessive amounts of tissue factor (either due to mechanical tissue injury and/or by endothelial and monocyte activation), leading to thrombin generation. Thrombin converts fibrinogen to fibrin monomers. In addition, thrombin is a potent agonist for platelet activation and aggregation. The above processes lead either to large vessel thrombosis, or more commonly, microvessel fibrin deposition, resulting in tissue ischemia and organ dysfunction. Furthermore, excess thrombin generation results in the proteolysis and depletion of coagulation factors, including fibrinogen, and factors II, V, VIII and X. The depletion of these factors is dependent of their plasma half-lives (Table 16.5) and the rate of synthesis by the liver, and can lead to bleeding. Moreover, thrombin induces endothelial cells to release tissue plasminogen activator (t-PA), which converts plasminogen to plasmin in the presence of the newly formed fibrin monomer. This results in fibrinolysis, which may lead to further consumption of coagulation factors, thus worsening bleeding. Finally, plasma levels of natural anticoagulants, including protein C, and antithrombin (III) are depleted during DIC. The laboratory abnormalities are enumerated in Table 16.9.

Therapy of DIC is aimed at prompt and aggressive management of the underlying condition. Other measures include initiation of basic support measures (e.g. correction of volume status, gas exchange, and electrolyte imbalance), determination whether replacement of platelets or clotting factors is necessary, and consideration whether institution of anticoagulants to stem the progression of DIC is needed.

Transfusion of platelets, fresh frozen plasma (FFP) and cryoprecipitate should be reserved for patients who have documented laboratory evidence of decompensated DIC and are bleeding, or are scheduled for

| Table 16.7: Conditions associated with disseminated intravascular coagulation |
|--------------------------------|--------------------------------|
| **Acute and subacute**       | **Chronic**                   |
| Infections                   | Malignancies (solid tumors)   |
| Gram-negative bacteria       | Obstetric complications       |
| Encapsulated gram-positive bacteria | Dead fetus syndrome         |
| Toxic shock syndrome         | Localized intravascular coagulation |
| Viruses (e.g. Varicella)     | Aortic aneurysm               |
| Obstetric complications      | Hemangiomas (Kasabach-Merritt syndrome) |
| Eclampsia/pre-eclampsia      | Advanced liver disease        |
| Abruptio placentae           | LeVeen shunt                  |
| Amniotic fluid embolism      | Fatty liver of pregnancy      |
| Sepsis                       |                               |
| Saline-induced abortion      |                               |
| Malignancies                 |                               |
| Leukemia, lymphoma           |                               |
| Tissue injury/crush injury   |                               |
| Burns                        |                               |
| Heat stroke                  |                               |
an invasive procedure. Platelets should be maintained over 20,000 to 30,000/mm³ in a bleeding patient, and greater than 50,000/mm³ in a patient undergoing a surgical procedure, or when there is significant blood loss. Cryoprecipitate should be administered to maintain a plasma fibrinogen > 100 mg/dl. FFP may be infused to correct prolonged PTT and INR in appropriate clinical circumstances. Replacement therapy may be required every 6 to 8 hours while the underlying cause of DIC is being treated.

Heparin infusion should be considered in DIC if the patient has thromboembolic manifestations of DIC (e.g. purpura fulminans, dead fetus syndrome before induction of labor, aortic aneurysm, malignancy associated DIC). Heparin should be used with caution as it may exacerbate DIC-associated bleeding.

Antithrombin (III) concentrates have been used in the treatment of DIC associated with sepsis. A meta-analysis or randomized clinical trials demonstrated its benefit in preventing organ failure and death. However, a recent large phase III trial of AT (III) in sepsis (KyberSept Trial) did not demonstrate any difference in 28-day all cause mortality between the AT (III) and placebo groups.

There is great interest in the use of activated protein C in sepsis-induced DIC. A randomized, double blind, placebo controlled clinical trial using recombinant human activated protein C (rhAPC) in patients with sepsis demonstrated a 6.1 percent absolute risk reduction in the 28-day all cause mortality (p = 0.005). Remarkably, the most critically ill patients derived the maximal benefit. The primary safety concern was the increased risk of bleeding in the rhAPC group; and for this reason (as well as the high cost of the product), it should be used judiciously. For a review, please see references.

### REFERENCES


The risk of venous thromboembolism is high following surgery, but it frequently can be reduced dramatically with appropriate prophylactic measures. If it does occur, its timely recognition and management is critical to prevent clot progression and to reduce its attendant risk of embolization. This chapter reviews aspects of the pathophysiology of thrombosis that are important to a surgeon. By way of definition, the term “hemostasis” is used in reference to the normal physiologic (and usually desirable) blood clotting response to breaching of blood vessel integrity. Thrombosis is a term that refers to the pathologic (and therefore undesirable) response of the coagulation system to some form of insult, whether recognized or not.

**PATHOPHYSIOLOGY OF THROMBOSIS**

Hemostasis is a complex interaction of platelets, coagulation factors, blood vessel walls and the inhibitory pathways. Please refer to the chapter “Hemorrhagic Coagulopathies in the Surgical Patient” for a detailed discussion of the physiology of hemostasis. Potential perturbation in any of the above-mentioned elements, either individually or collectively, may give rise to thrombosis in the postoperative period. These perturbations can either be hereditary or acquired. Preoperative detection of these defects is the key to minimizing potential complications. A careful history and physical examination more often than not will offer clues to a patient’s thrombotic risk.

**POSTSURGICAL VENOUS THROMBOEMBOLISM**

Surgery is a major risk factor for venous thromboembolism (VTE). Virchow’s triad of venous stasis, vessel wall injury and hypercoagulability are germane to a postsurgical patient’s risk for VTE. Surgery itself is thrombogenic for a variety of reasons:

1. Release of tissue factor following incision and tissue injury;
2. Use of general anesthesia and paralytic agents;
3. Bedrest and limb immobilization, leading to lack of lower leg muscle activity; and
4. Decreased fibrinolytic activity postoperatively.

Some thrombotic complications can be anticipated preoperatively through a careful medical history (including a personal and family history of arterial and venous thrombosis) and physical examination. Some of the known risk factors that predispose to VTE are listed in Table 17.1. Moreover, certain surgical procedures, such as hip and knee replacement surgery, and pelvic surgery inherently carry a high risk of thrombotic complications, and VTE prophylaxis should be routinely administered. The risk of venous thrombosis varies with the type of surgery and patient characteristics; therefore, it is important to stratify the risk of VTE so that aggressive prophylactic measures can be instituted in high-risk patients (Table 17.2). This strategy also minimizes the potential adverse effects of anticoagulation in subgroups with a low risk of VTE.
Thrombotic Disorders in the Surgical Patient

Postoperative VTE can manifest as either acute symptomatic DVT or pulmonary embolism (PE). Apart from the short-term risk of death from PE, DVT is associated with a significant long-term morbidity. The 8-year cumulative incidence of post-thrombotic syndrome, the symptoms of which include chronic leg swelling, stasis dermatitis, skin ulceration and pain, is approximately 30 percent. In addition, the persistence of residual vein thrombus following therapy increases the odds of recurrent episodes of DVT.

PREVENTION OF VENOUS THROMBOEMBOLISM

Early ambulation of a postsurgical patient is key to reduce venous stasis, and thus, to reduce the incidence of VTE. Graduated compression stockings or pneumatic boots are good tools to prevent venous stasis in the lower extremity while a patient is bedridden. Full anticoagulation with heparin is associated with a high incidence of postoperative bleeding complications, and is inappropriate for prophylactic use except in patients with a recent history of VTE. Perioperative

### Table 17.1: Hypercoagulable states

<table>
<thead>
<tr>
<th>Heredity</th>
<th>Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden mutation (activated protein C resistance)</td>
<td>Anticardiolipin antibody and/or lupus anticoagulant</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>Therapeutic agents (L-asparaginase, mitomycin, tamoxifen, raloxifene, prothrombin complex concentrates)</td>
</tr>
<tr>
<td>Antithrombin III deficiency</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dysfibrinogenemia</th>
<th>Immobilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperhomocysteinemia</td>
<td>Trauma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surgery/postoperative state</th>
<th>Pregnancy and postpartum period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor surgery in patients aged 40-60 years or major surgery in patients &lt;40 years of age with no additional risk factors</td>
<td>Malignancy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nephrotic syndrome</th>
<th>Paroxysmal nocturnal hemoglobinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperhomocysteinemia</td>
<td>Myeloproliferative syndromes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Patients</th>
<th>Approximate risk (without prophylaxis)</th>
<th>Recommendations for prevention of venous thromboembolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Minor surgery in patients under age 40 with no additional risk factors; surgery lasting &lt;30 min;</td>
<td>2%</td>
<td>Early and aggressive mobilization, leg exercises</td>
</tr>
<tr>
<td>Moderate</td>
<td>Nonmajor surgery in patients aged 40-60 years or major surgery in patients &lt;40 years of age with no additional risk factors; minor surgery with additional risk factors</td>
<td>10–20%</td>
<td>LDUF q12h, LMWH, graded compression elastic stockings, or intermittent pneumatic compression</td>
</tr>
<tr>
<td>High</td>
<td>Nonmajor surgery in patients &gt;60 yr; major surgery in patients above 40, or additional risk factors</td>
<td>20–40%</td>
<td>LDUF q8h, LMWH, or intermittent pneumatic compression</td>
</tr>
<tr>
<td>Highest</td>
<td>Major surgery in patients &gt; 40 yr plus history of previous VTE, cancer, molecular hypercoagulable state, hip fracture surgery, hip or knee arthroplasty, major trauma</td>
<td>40–80%</td>
<td>LMWH, fondaparinux, warfarin, or intermittent pneumatic compression + LDUH/LMWH</td>
</tr>
<tr>
<td>Special situations</td>
<td>Neurosurgery or other patients with high bleeding risk</td>
<td>Open prostatectomy</td>
<td>Intermittent pneumatic compression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intermittent pneumatic compression</td>
</tr>
</tbody>
</table>

LDUH: low-dose unfractionated heparin; LMWH: low molecular weight heparin

Adapted from the seventh ACCP Consensus Conference on antithrombotic therapy; Chest: 126 (Suppl 3); 2004.
administration of low-dose unfractionated heparin reduces the incidence of deep vein thrombosis by 60 to 80 percent for general surgery. The utility of oral anticoagulation with warfarin is limited by the frequency of bleeding complications, the relative difficulty in achieving stable therapeutic values, and the delay in achieving sufficient anticoagulation. If, however, 4 to 6 weeks of VTE prophylaxis is being considered, especially for orthopedic surgery, warfarin is a good option. A target INR maintained between 2.0 and 3.0 is considered to provide adequate anticoagulation. Other agents such as low-molecular-weight heparins (LMWH), heparinoids, and pentasaccharides (Fondaparinux) have also been used successfully (Table 17.3). Although the consensus recommendations published by the 6th American College of Chest Physicians (ACCP) consensus conference are to initiate prophylactic doses of anticoagulation 1 to 12 hours prior to the surgery,4 typically in the US, the prophylactic therapy is started 6 to 12 hours post-operatively, once adequate hemostasis has been achieved. In fact, as there are only small differences in outcomes between initiating LWMH for major orthopedic surgical procedures pre- or post-operatively, the 7th ACCP consensus conference recommends that both strategies are acceptable.1

Patients on long-term anticoagulation (for example, prosthetic heart valves, chronic atrial fibrillation, antiphospholipid antibody syndrome, history of recurrent VTE) need to be switched from oral warfarin to high-dose intravenous heparin or therapeutic doses of subcutaneous LMWH about 4 to 5 days prior to elective surgery. The dose of LMWH should be held at least 12 hours prior to the procedure (2 hours for the UFH), and re-started 8 to 12 hours after the surgery, once adequate hemostasis is achieved. Warfarin can be reinitiated once the patient resumes a normal diet, and heparin/LMWH overlapped with it for at least 2 days after the INR is above 2.0. For an emergent surgical procedure, the effect of warfarin may be reversed by administering 1 to 2 mg of vitamin K orally or intravenously, along with infusion of fresh frozen plasma, to reduce the INR below 1.4. High doses of vitamin K are usually unnecessary and may lead to prolonged resistance to subsequent re-anticoagulation with warfarin.

TREATMENT OF VENOUS THROMBOEMBOLISM

The threshold for screening patients manifesting symptoms consistent with DVT (e.g. leg swelling, pain, and tenderness on palpation of the calf muscles) or PE (e.g. pleuritic chest pain, shortness of breath, hemoptysis, and palpitations) should be low in the postsurgical setting. Venous duplex/ultrasound has a high sensitivity and specificity in diagnosing proximal DVT, and has obviated the routine use of venography. A ventilation/perfusion lung scan or a high-resolution chest CT with pulmonary angiogram are the diagnostic studies to consider if PE is suspected. Of note, high-resolution CT angiograms are excellent to diagnose PE extending out to the segmental branches of the pulmonary vasculature, but lose sensitivity in more peripheral vessels. If DVT or PE is confirmed, patients should be initiated on

<table>
<thead>
<tr>
<th>Class</th>
<th>Agent</th>
<th>Dose</th>
<th>Route and frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated heparin</td>
<td>Heparin</td>
<td>5000 units</td>
<td>SC q8-12h</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>75-100 units/kg</td>
<td>SC q12h</td>
</tr>
<tr>
<td>LMWH</td>
<td>Enoxaparin</td>
<td>30 mg</td>
<td>SC q12h</td>
</tr>
<tr>
<td></td>
<td>Enoxaparin</td>
<td>40 mg</td>
<td>SC daily</td>
</tr>
<tr>
<td></td>
<td>Dalteparin</td>
<td>5000 units</td>
<td>SC daily</td>
</tr>
<tr>
<td></td>
<td>Tinzaparin</td>
<td>75 units/kg</td>
<td>SC daily</td>
</tr>
<tr>
<td></td>
<td>Tinzaparin</td>
<td>3500-4500 units</td>
<td>SC daily</td>
</tr>
<tr>
<td>Heparinoid</td>
<td>Danaparoid</td>
<td>750 units</td>
<td>SC q12h</td>
</tr>
<tr>
<td>Pentasaccharide (Xa inhibitor)</td>
<td>Fondaparinux</td>
<td>2.5 mg</td>
<td>SC daily</td>
</tr>
<tr>
<td>Vitamin K antagonist</td>
<td>Warfarin</td>
<td>5 mg</td>
<td>PO daily. Adjust dose to maintain INR between 2.0-3.0 SC q12h</td>
</tr>
<tr>
<td>Thrombin inhibitor</td>
<td>Desirudin (recombinant Hirudin)</td>
<td>15 mg</td>
<td></td>
</tr>
</tbody>
</table>

LMWH: low-molecular-weight heparin
intravenous heparin, after a baseline CBC, aPTT and INR is obtained. A bolus of 80 units/kg heparin should be administered, and a continuous infusion at 18 units/kg/hr initiated. APTT should be monitored every 6 hours, and maintained 1.5 to 2.5 times the mean normal value. Additionally, a platelet count should be monitored at least every other day while a patient is on heparin to observe for the possible development of heparin associated thrombocytopenia (see below for details). Oral warfarin at a dose of 5 mg daily should be initiated along with heparin if the patient has resumed eating. Concomitant heparin and warfarin should be continued for at least 5 days; when the INR has been greater than 2.0 on two consecutive days, the heparin may be discontinued. Subcutaneous low-molecular-weight heparin (LMWH) or pentasaccharides may be substituted for intravenous heparin if the creatinine clearance is within normal limits, but it should be noted that the half-life of these agents is longer than unfractionated heparin, and protamine sulfate is not as effective an antidote for LMWH or pentasaccharides in the event that bleeding complications ensue. Usually, three months of therapy with warfarin is adequate for an episode of postsurgery DVT. For additional reading, please see reference.5

HEPARIN-ASSOCIATED THROMBOCYTOPENIA

Heparin is used frequently in the surgical intensive care unit. It is used as an anticoagulant for intra-arterial catheters, central venous access catheters, hemodialysis, and for perioperative prophylaxis against VTE.

Heparin-associated thrombocytopenia (HAT) develops in 1 to 3 percent of patients treated with unfractionated heparin. It is typically associated with mild-to-moderate thrombocytopenia (40,000–70,000/mm³), although platelet counts may occasionally fall as low as 20,000/mm³. However, spontaneous bleeding is rare. Instead, localized or disseminated venous or arterial thrombosis may occur in 30 to 50 percent of patients with HAT. Thrombocytopenia (+/- thrombosis) typically manifests 5 to 14 days after the initiation of heparin therapy.6,7

HAT is caused by a heparin dependent IgG antibody that recognizes a complex of heparin and platelet factor-4 (PF-4). These immune complexes activate platelets via platelet Fc receptors, leading to intravascular platelet aggregation and thrombocytopenia, and generation of thrombin.8 Furthermore, tissue factor is expressed on endothelial cells that are activated by the HAT antibody that may recognize PF-4 bound to endothelial heparan sulfate. The above processes may result in arterial or venous thrombosis. The immunogenicity and the platelet activating effects of heparin are proportional to its molecular size and degree of sulfation; thus, unfractionated heparin is more likely to cause HAT compared to low-molecular-weight heparin (LMWH) or pentasaccharides. Other factors influencing the development of HAT include previous exposure to heparin, and heparin from a bovine rather than porcine source. Only a minority of patients who generate HAT antibodies develop thrombocytopenia and thrombosis. Moreover, clinical factors play a role in determining the sequela of HAT. For example, postoperative orthopedic patients are at high risk for venous thrombosis, whereas postoperative cardiovascular patients are at relatively high risk for arterial thrombosis.9

In the laboratory, an enzyme-linked immunosorbent assay (ELISA) utilizing heparin/PF-4 as the target antigen may be used to detect the pathogenic IgG in HAT. The assay for heparin-dependent platelet antibody detection by platelet aggregometry has high specificity, but low sensitivity. It should be noted that the clinical history—including the timing of onset of thrombocytopenia—is the most important clue to the diagnosis of HAT. The “gold standard” for diagnosis is the ¹⁴C serotonin platelet release assay, which has >95 percent sensitivity and specificity, although it is available in relatively few institutions.

Once HAT is suspected, heparin from all possible sources should be discontinued. It is generally recommended that even in the absence of overt arterial or venous thrombosis, systemic anticoagulation with intravenous recombinant Hirudin (Lepirudin), Argatroban, or danaparoid should be considered (Table 17.4). Hirudin and Argatroban are direct thrombin inhibitors and do not have any crossreactivity with the HAT antibody. Danaparoid has 10 to 40 percent in vitro crossreactivity with HAT antibody; however, clinically significant crossreactivity is uncommon. Low-molecular-weight heparins (LMWH) should not be substituted for unfractionated heparin when HAT is suspected, as there may be
significant crossreactivity between the antibody and LMWH. Pentasaccharides and Bivalirudin have been used with no reported complication in the management of HAT, although these agents do not have this specific indication for their use. Warfarin should not be initiated in the absence of a rapid acting systemic anticoagulant, as it depletes the regulatory protein C first (due to its short half-life), thus exacerbating thrombosis.

To monitor for the development of HAT, measuring of the platelet count on alternate days (for at least 14 days) in all patients receiving heparin is recommended, regardless of the dose. Although LMWHs are less immunogenic as compared to unfractionated heparin, periodic checks on the platelet count is also recommended.

REFERENCES


Plasma is the liquid part of blood that contains factors and inhibitors participating in the coagulation process. It also contains water, electrolytes, albumin, immunoglobulins and other proteins. Depending on a number of criteria including collection and storage specifications, a variety of plasma products can be prepared for therapeutic use. This chapter discusses fresh frozen plasma and related plasma components. Table 18.1 briefly summarizes their salient features.

**PRODUCTS, PREPARATION AND STORAGE**

Plasma can be obtained for therapeutic use by processing donated whole blood or through an apheresis procedure. It needs to be stored in frozen form to preserve the clotting factor activity.

**Fresh Frozen Plasma, Frozen Plasma and Thawed Plasma**

In order to meet the criteria for fresh frozen plasma (FFP), plasma has to be separated and frozen at −18°C or colder within six to eight hours of whole blood collection. On average, FFP contains 1 U/ml of all the clotting factors and 200-450 mg/dl of fibrinogen. On the other hand, frozen plasma (FP) is separated and frozen between 8 and 24 hours after phlebotomy. The level of the labile clotting factors, factors VIII and V, is lower in FP, than in FFP.

Plasma may be frozen rapidly either in a dry ice-ethanol or in a dry ice-antifreeze bath, or between layers of dry ice, or in a blast freezer or in a mechanical freezer kept at temperatures of −65°C or lower. If a liquid bath is used, plasma should be overwrapped with a plastic bag to protect the unit from damage. Different methods are recommended for easy detection of inadvertent thawing and subsequent refreezing of the unit. One such method is freezing the plasma in a horizontal position but storing it upright. The movement of the air bubbles in the unit will indicate if it has been thawed. Alternatively, a tube can be pressed into the bag or a rubber band can be placed around the liquid plasma bag during freezing. The subsequent disappearance of the indentation caused by the tube or the rubber band will indicate that thawing has occurred.

After freezing, FFP can be stored at −18°C for up to one year. If frozen and maintained at −65°C, FFP can be stored for up to 7 years. For transfusion, it is thawed over 20 to 30 minutes at 30 to 37°C and then stored at 4°C (1–6°C). Usually, a water bath is used for thawing. The entry ports need to be protected from contamination during this process. This can be achieved by putting the unit in a plastic overwrap or by positioning and securing the unit in an upright position so that entry ports stay above the water level.

The 2002 Circular of Information for the Use of Human Blood and Blood Components (American Association of Blood Banks, American Red Cross, America’s Blood Centers) describes FFP as “containing
<table>
<thead>
<tr>
<th>Name of product</th>
<th>Description</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh frozen plasma (FFP) 1,3,7,11</td>
<td>Plasma frozen within 6–8 hours of phlebotomy. Stored at ≤ −18°C for up to one year, or at ≤ −65°C for up to 7 years</td>
<td>Thawed plasma contains reduced levels of labile coagulation factors compared with FFP</td>
</tr>
<tr>
<td>FFP thawed 2,11</td>
<td>Once thawed, FFP is stored at 1–6°C and called FFP Thawed for the first 24-hour post-thaw period</td>
<td></td>
</tr>
<tr>
<td>Frozen plasma (FP) 1,11</td>
<td>Plasma frozen within 24 hours of phlebotomy. Stored at ≤ −18°C for up to one year</td>
<td></td>
</tr>
<tr>
<td>Thawed plasma 2,11</td>
<td>FFP that has been thawed but not transfused within first 24 hours post-thaw Stored at 1–6°C and transfused for up to 5 days post-thawing</td>
<td></td>
</tr>
<tr>
<td>Plasma cryoprecipitate reduced 2,11 (cryosupernatant)</td>
<td>Plasma depleted of the cryoprecipitate fraction and then refrozen Stored at ≤ −18°C for up to 12 months after original collection</td>
<td>It may be used for treatment of TTP</td>
</tr>
<tr>
<td>Plasma: liquid plasma 2</td>
<td>Can be separated from whole blood unit for up to 5 days after the expiration date of the whole blood. If not frozen but stored at 1–6°C it is called liquid plasma and can be used for up to 5 days after the expiration date of the whole blood unit. If stored frozen at −18°C or lower, it is called plasma and can be stored and used for up to 5 years after the date of collection. FFP that has not been used within a year can also be designated as “plasma”, and appropriately relabeled and maintained frozen, can be used for an additional 4 years</td>
<td></td>
</tr>
<tr>
<td>Solvent/detergent-treated plasma 2,3,7,11</td>
<td>Plasma product derived after a pathogen inactivation process. Significantly inactivates the lipid enveloped viruses but not the nonlipid enveloped viruses. Stored at ≤ −18°C for up to 24 months from manufacture</td>
<td></td>
</tr>
<tr>
<td>FFP, methylene blue and light-treated (MBFFP), and FFP, methylene blue-treated and removed 6,10</td>
<td>Plasma products derived after a pathogen inactivation process</td>
<td>FVIII:C content reduced when compared to standard FFP</td>
</tr>
<tr>
<td>FFP donor retested 2</td>
<td>FFP stored for 112 days or longer. The product is released only if the donor is then retested and continues to be negative for transfusion-transmitted diseases</td>
<td>This is presumed safer than FFP since concerns about the donor being in an infectious window period at donation are alleviated</td>
</tr>
<tr>
<td>Linked FFP 2</td>
<td>The FFP unit is “linked” to the RBC unit from the same donation, so that a patient needing both RBC and FFP transfusion can receive the “linked FFP” from the same donation as the RBC unit</td>
<td>A patient receiving both the RBC and the “linked FFP” from the same donation has no increased risk of exposure from the FFP. No benefit if only FFP transfusion is needed</td>
</tr>
<tr>
<td>Recovered plasma 2,11</td>
<td>It is prepared from “plasma” or “liquid plasma”. Stored at ≤ −18°C</td>
<td>In the US it is usually shipped to fractionators, for processing into derivatives</td>
</tr>
<tr>
<td>Source plasma; single donor plasma 1,7</td>
<td>It is obtained by plasmapheresis of a single donor. Stored at −20°C after collection</td>
<td></td>
</tr>
</tbody>
</table>
functional amounts of labile coagulation factors (Factors V and VIII). After thawing, FFP should be transfused immediately or stored at 1 to 6°C for up to 24 hours, up to which time it is called “FPF thawed”. After this time, the words “fresh frozen” should be removed because of the reduction of the labile factors, and it should be labeled as “thawed plasma”. Recent studies show that the significant difference in plasma frozen at 24 hours after whole-blood donation compared to freezing within 8 hours is a decreased level of factor VIII.4 For this reason, some experts believe that for most therapeutic indications (except for situations requiring only factor VIII replacement), thawed plasma is therapeutically equivalent to and interchangeable with FFP or FP.4,8 Since factor VIII is an acute phase reactant, its level is elevated in many disease states and the mild decrease of factor VIII level in thawed plasma is not clinically significant.8 For isolated factor VIII replacement virally inactivated or recombinant factor VIII concentrates, not plasma infusions, are recommended.4,9 Using thawed plasma has the advantage of more timely release from the Blood Bank since it is already in the liquid form and ready for transfusion. It also decreases wastage of FFP, as it can be used for up to 5 days after thawing rather than being discarded after 24 hours.8

Plasma Cryoprecipitate Reduced (Cryosupernatant)

When FFP is thawed in a 1°C and 6°C environment, a cold insoluble material can be separated to obtain cryoprecipitate.3 The remaining thawed plasma is called cryoprecipitate reduced (cryo-poor) plasma or cryosupernatant. When stored at –18°C or colder, it has an expiration date of one year from the whole blood collection date.2 Since cryoprecipitate contains fibrinogen, factor VIII, von Willebrand’s factor (vWF), factor XIII and fibronectin, these factors are reduced in the cryo-poor plasma. Cryo-poor plasma may be used for treatment of refractory thrombotic thrombocytopenic purpura, since cryo-poor plasma is deficient in the pathogenic ultra-large multimers of vWF but contains vWF-cleaving metalloprotease that the patient needs.6

Plasma: Liquid Plasma

Plasma in a whole-blood unit can be separated and removed at any time during storage, for up to five days after the expiration date of the whole blood. If stored at 1 to 6°C, it is called liquid plasma and can be used for up to five days after the expiration date of the whole blood from which it was made. If stored frozen at –18°C or lower, it is called plasma and can be stored and used for up to five years after the date of collection. FFP that has not been used within a year can also be designated as “plasma”, and stored frozen and suitably relabeled, can be used for an additional 4 years. Since potassium and ammonia levels increase during storage of red blood cells, plasma prepared from outdated blood differs from plasma initially prepared as FFP.2 In the USA, liquid plasma can be fractionated to produce other plasma derivatives such as albumin.1

Solvent/detergent-treated Plasma

Solvent/detergent-treated plasma (S/D plasma)2,3,7 is prepared from pools 2,500 or fewer units of ABO type specific plasma that have been frozen to preserve the labile clotting factors. The thawed plasma is treated with solvent tri-n-butyl phosphate (1%) and detergent Triton X-100 (1%). The solvent and detergent are removed after treatment and the sterile-filtered plasma is refrozen.2,3 This process significantly inactivates the lipid enveloped viruses (e.g. HCV, HBV, HIV), but not the nonlipid enveloped viruses such as hepatitis A and parvovirus.2,3,7 The product contains a minimum of 0.7 U/ml of factors V, VII, X, XI, and XIII and a minimum of 1.8 mg/ml of fibrinogen. It lacks the largest multimers of vWF, and some coagulation inhibitors are decreased.2 It was provided as 200 ml units, but is no longer available in the US.

FFP, Methylene Blue-treated (MBFFP), and FFP, Methylene Blue-treated and removed6,10

These plasma components are also pathogen-reduced. Inactivation of pathogens is carried out by treatment with methylene blue followed by exposure to visible light. Adsorption filtration may then be used to remove a significant amount of methylene blue. The treatment process reduces the FVIII:C content by approximately 30 percent when compared to standard FFP.10 It is provided as units of single donor plasma.6
CLINICAL USES

Fresh frozen plasma is useful when multiple factor deficiencies need to be corrected simultaneously or when there is no factor concentrate available to correct an isolated factor deficiency. FFP should not be used when more effective or safer alternatives are available. In patients with an inhibitor to a specific factor it is unlikely to provide significant benefit. In most mild or moderate coagulopathies, doses in the range of 10 to 15 ml/kg have been recommended. However, the dose, frequency and duration of therapy should be based on the clinical indication, desired hemostatic level and relevant coagulation tests, as well as response to therapy. The volume required may sometimes become a limiting factor. Plasma infusions need to be type specific or ABO compatible.

Coagulopathy due to Multiple Factor Deficiencies, with Bleeding or before Invasive/surgical Procedures

A number of clinical conditions can be associated with deficiency of multiple coagulation factors. FFP may be indicated in such cases if associated with bleeding, or before invasive procedures. Therapeutic decisions should not be based on laboratory results alone. It is also necessary to take into account the patient’s clinical disorder, physiological status and co-morbid conditions, as well as the extent of active bleeding and the type of invasive procedure planned. Therefore, clinical correlation is essential and recommendations can only serve as general guidelines. Some common clinical conditions are discussed below.

Liver Dysfunction with Significant Coagulation Abnormalities

Coagulopathy associated with liver dysfunction is often multifactorial in etiology. Vitamin K deficiency is one of the correctable contributors to coagulopathy, and a trial of vitamin K replacement is, therefore, important. FFP may be used in the setting of active bleeding, or before invasive procedures. Some experts have addressed the common policy of performing a liver biopsy only if the PT is no more than 4s above the upper limit of the normal range. They concluded there is “no evidence to substantiate this”, and that more studies are needed to establish the role of FFP in correcting bleeding tendency before liver biopsy. If FFP infusion is carried out it is important to check post-infusion laboratory results to guide therapy. They also questioned the routine use of FFP to prevent bleeding in patients with liver disease and elevated PT, since complete normalization of coagulopathy may not occur with plasma transfusion, and the extent of response is unpredictable.

Warfarin-induced Coagulopathy

Vitamin K is required for the post-translational modification of some coagulation proteins (Factors VII, II, IX, and X) and regulatory proteins C and S. This modification is essential for the activity of these coagulation proteins. Warfarin blocks the recovery of active form of vitamin K and renders these proteins inactive. In most situations, where reversal of warfarin is necessary, withholding the drug and administration of vitamin K is sufficient. FFP is indicated for patients on warfarin therapy when there is active bleeding, or when an emergent invasive procedure is required and time is not sufficient to wait for the effect of vitamin K therapy. Plasma infusion may have only a sub-optimal effect. In case of massive overanticoagulation with warfarin, the volume of FFP required (up to 15–20 ml/kg) may be of concern. If there are no contraindications, prothrombin complex concentrates (PCC) with a higher concentration of vitamin K-dependent factors may be preferable to FFP.

Dilutional Coagulopathy

Massive transfusion, defined as replacement of one blood volume or more in 24 hours, can cause dilutional coagulopathy. These patients may have prolonged PT and PTT combined with thrombocytopenia. Some coagulation factors can decrease below hemostatic levels, contributing to increased bleeding tendency. FFP may be infused in this setting when abnormal laboratory results coexist with significant coagulopathy (e.g. bleeding when PT/ APTT is greater than 1.5 times midrange normal, and fibrinogen has been corrected to greater than 80–100 mg/dl). In the perioperative setting, the effect of residual heparin should be taken into account and corrected if possible. Plasma therapy should be guided by the clinical picture and timely coagulation tests rather than by a fixed replacement formula.
Platelet count should be maintained above $50 \times 10^9/L$. Cryoprecipitate may be considered if fibrinogen level is below 100 mg/L and the predominant aim is to correct hypofibrinogenemia.

**Disseminated Intravascular Coagulation**

In disseminated intravascular coagulation (DIC) the activation of the coagulation system leads to diffuse thrombin formation and consumption of procoagulant factors and platelets. Bleeding tendency may be exacerbated by hyperfibrinolysis. A wide variety of disorders can lead to DIC and treatment of the underlying cause is crucial. Therapy needs to be tailored to the individual, based on clinical symptoms with the help of laboratory results. Active bleeding and planned invasive procedure are common indications for transfusion therapy. Red blood cells, FFP, platelet concentrates, and fibrinogen replacement with cryoprecipitate may be needed for actively bleeding patients. PT, PTT, fibrinogen level and platelet count should be monitored at short intervals (4–6 hours) to guide therapy. Low-dose heparin may be recommended in cases when thrombosis dominates the clinical picture. The risk of bleeding in this setting, as well as the possible decrease in efficacy of heparin due to decreased ATIII should be considered when making this decision.

**Selected Single Factor Deficiencies**

Fresh frozen plasma should not be used when “virus-safe” products are available for replacement of single factor deficiency. Availability of products such as virally inactivated or recombinant factor concentrates varies in different parts of the world. Specific factor concentrates that may be unavailable in some parts of the world include Factors II, V, X, XI or XIII. If FFP has to be used in such a situation, the initial dose is usually in the range of 15 to 20 ml/kg, followed by 3 to 6 ml/kg. Factor XIII replacement, however, requires a much smaller dose. The minimum level required for hemostasis varies for different factors. The frequency of administration depends on the half-life of the specific factor being replaced. Factor VII has the shortest half-life among the clotting factors (3–6 hours) and factor XIII has the longest (9 days).

**Thrombotic Thrombocytopenic Purpura**

Thrombotic thrombocytopenic purpura (TTP) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever combined with neurological and renal dysfunction. Often the underlying abnormality is the deficiency of, or the presence of neutralizing antibodies against, ADAMTS 13 metalloprotease enzyme. The standard therapy is plasma exchange with FFP as a replacement fluid to remove the neutralizing antibody and to supply the missing or dysfunctional cleaving protease. Cryosupernatant plasma lacking the ultra-large vWF multimers may be recommended in refractory cases.

**Other Indications**

Fresh frozen plasma has been used, and recommended in the setting of C1 esterase inhibitor deficiency with acute angioedema or for preoperative prophylaxis. It has also been used in ATIII, protein C or S deficiency. However, the availability of specific concentrates will likely limit the use of plasma in some of these settings (Table 18.2).

**Table 18.2: Overview of common indications for plasma infusion therapy**

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulopathy caused by multiple factor deficiencies, when the patient is bleeding, or needs an emergent invasive procedure.</td>
</tr>
<tr>
<td>- Liver dysfunction</td>
</tr>
<tr>
<td>- Warfarin induced coagulopathy</td>
</tr>
<tr>
<td>- Dilutional coagulopathy</td>
</tr>
<tr>
<td>- Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>Coagulopathy caused by selected single factor deficiencies</td>
</tr>
<tr>
<td>- Should be used only if specific factor concentrates are not available.</td>
</tr>
<tr>
<td>Thrombotic thrombocytopenic purpura (TTP)</td>
</tr>
<tr>
<td>- Used for plasma exchange.</td>
</tr>
<tr>
<td>Rare plasma protein deficiencies</td>
</tr>
<tr>
<td>- C1 esterase inhibitor deficiency</td>
</tr>
<tr>
<td>- Should be used only if specific concentrates are not available.</td>
</tr>
</tbody>
</table>

**CONTRAINDICATIONS AND PRECAUTIONS**

Plasma should not be used for the sole purpose of volume expansion, or as a nutritional supplement. It should not be used routinely to reverse vitamin K
Handbook of Blood Banking and Transfusion Medicine

deficiency; exceptions to this in the setting of warfarin anticoagulation have already been discussed. When coagulopathy is more effectively and/or safely corrected with specific therapies such as vitamin K, factor concentrates or cryoprecipitate, they are preferred.

Plasma is not recommended for immunoglobulin replacement therapy. Its use in cases of massive red cell transfusion or after cardiopulmonary bypass should not be prophylactic but based on laboratory results and clinical assessment.

Immunoglobulin (IgA) deficient patients with antibodies to IgA are at risk of anaphylaxis on exposure to IgA, and should receive plasma from IgA-deficient donors.

**Adverse Effects**

Adverse events in the most part are not unique to plasma products but mirror the adverse events related to any blood product transfusion. Because of the paucity of WBCs, some adverse events are less likely to occur with acellular FFP than with cellular blood products. These include the risk of CMV and HTLV transmission. To date, transfusion-associated GVHD has not been reported from transfusion of FFP. There is some concern about the risk of thromboembolism with the use of S/D FFP, that has reduced coagulation inhibitors, for plasma exchange in TTP. Potential adverse events linked to FFP transfusion are summarized in Table 18.3. Reactions observed with plasma transfusions are depicted Table 18.4.

**Table 18.3: Theoretical adverse effects of plasma transfusion**

<table>
<thead>
<tr>
<th>Complication Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunologic complications</td>
<td>Allergic reactions ranging from urticaria to anaphylaxis (rule out IgA deficiency)</td>
</tr>
<tr>
<td></td>
<td>Febrile nonhemolytic transfusion reaction</td>
</tr>
<tr>
<td></td>
<td>Transfusion-related acute lung injury</td>
</tr>
<tr>
<td></td>
<td>Alloimmunization to platelets, Rh(D), other RBC antigens, exogenous antigens</td>
</tr>
<tr>
<td></td>
<td>Immunomodulation/immunosuppression</td>
</tr>
<tr>
<td></td>
<td>Isoagglutinins causing hemolysis or positive DAT</td>
</tr>
<tr>
<td></td>
<td>Thromboembolism: concern with use of S/D FFP</td>
</tr>
<tr>
<td></td>
<td>Post-transfusion purpura</td>
</tr>
<tr>
<td></td>
<td>Transfusion Associated GVHD: To date, not reported with FFP</td>
</tr>
<tr>
<td>Infectious complications</td>
<td>Bacterial contamination</td>
</tr>
<tr>
<td></td>
<td>Viral diseases: HIV, HBV, HCV, HAV, EBV, HHV-8, prions</td>
</tr>
<tr>
<td></td>
<td>WBC associated: CMV, HTLV I/II—no report with FFP</td>
</tr>
<tr>
<td></td>
<td>Others: Malaria—no report with FFP</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Donor specific such as medications, infections</td>
</tr>
<tr>
<td></td>
<td>Processing or storage related: cytokines, anaphylatoxins, preservatives</td>
</tr>
<tr>
<td>Physicochemical factors related to plasma infusion</td>
<td>Volume overload</td>
</tr>
<tr>
<td></td>
<td>Hypothermia: A warmer should be considered if large volumes are to be rapidly infused</td>
</tr>
<tr>
<td>Metabolic complications</td>
<td>Citrate toxicity with hypocalcemia, especially in patients with liver disease or circulatory collapse</td>
</tr>
<tr>
<td></td>
<td>Hypo/Hyperkalemia, acidosis, alkalosis</td>
</tr>
</tbody>
</table>

**Table 18.4: Observed reactions during plasma transfusions**

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaphylactic reactions</td>
<td>Urticaria, bronchospasm, angioedema, hypotension: allergic reaction to unknown protein</td>
</tr>
<tr>
<td></td>
<td>Anti-IgA in IgA-deficient recipient</td>
</tr>
<tr>
<td></td>
<td>Anti-C4: anti-Chido (C4d), anti-Rodgers(C4d) in recipient</td>
</tr>
<tr>
<td></td>
<td>Anti-haptoglobin antibodies in recipient deficient of haptoglobin</td>
</tr>
<tr>
<td>Acute pulmonary reactions</td>
<td>Transfusion-related acute lung injury: donor WBC antibody incompatible with recipient</td>
</tr>
<tr>
<td></td>
<td>Circulatory volume overload</td>
</tr>
<tr>
<td>Acute hemolysis</td>
<td>Anti-A (group O donor) infused to group A recipient</td>
</tr>
<tr>
<td>Acute hypotension</td>
<td>Bradykinin generation by filter, recipient taking ACE inhibitor</td>
</tr>
<tr>
<td>Severe acute thrombocytopenia</td>
<td>Donor platelet-specific antibody incompatible with recipient</td>
</tr>
<tr>
<td></td>
<td>Post-transfusion purpura</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>From the citrate during massive transfusions</td>
</tr>
</tbody>
</table>
concentrate; α1AT concentrate; or PCC. If only fibrinogen replacement is required, cryoprecipitate may be preferred. As with all blood products, plasma transfusion should be utilized based on a clear clinical indication, and with an understanding of attendant risks and benefits.

REFERENCES

Cryoprecipitate is a cold-insoluble fraction of plasma proteins contained in fresh frozen plasma (FFP). It is prepared by centrifugation of FFP after thawing at a temperature between 1 and 6°C. After immediate separation from the plasma, cryoprecipitate is refrozen within one hour and stored at −18°C or colder for up to twelve months from the date of blood collection. Cryoprecipitate is a low-volume blood component of approximately 5 to 20 ml per unit depending mainly on the amount of supernatant retained.

After Pool and Shannon described a method to produce cryoprecipitate from whole blood in a sterile closed bag system in 1965, it was produced by blood banks as the main source of concentrated coagulation factor VIII for the treatment of hemophilia A. Its official name is cryoprecipitated antihemophilic factor due to the discovery of its usefulness in providing factor VIII to patients with hemophilia A. Cryoprecipitate has now been replaced for this indication by commercially-prepared virus-inactivated or recombinant factor VIII concentrates in many countries and should no longer be used if a safer product is available. The factor VIII content in cryoprecipitate not only depends on the preparation technique and age of plasma used, but it is higher in blood group A than in group O donors and if citrate-phosphate-dextrose (CPD) rather than acid-citrate-dextrose (ACD) is used as anticoagulant. According to AABB standards, a unit of cryoprecipitate must contain at least 80 IU of factor VIII.

The important constituents of cryoprecipitate are fibrinogen, von Willebrand’s factor (vWF), factor VIII, factor XIII and fibronectin (Table 19.1). Cryoprecipitate cannot serve as a therapeutic source of the coagulation factors II, V, VII, IX, X, XI and XII. These need to be replaced by other products such as FFP or specific concentrates. The transfusion of cryoprecipitate as a source of fibronectin, a mediator of tissue repair and contained in cryoprecipitate, has not shown beneficial effects in critically ill patients. Recovery of plasma proteins in cryoprecipitate is approximately 40 to 70 percent of vWF and 20 to 30 percent factor XIII in the original unit of FFP. The fibrinogen content is on average 250 mg per unit with a minimum of 150 mg per unit according to AABB standards. Variations of the amount of these proteins, however, can be considerable, not only between single units but also between blood banks, so that typical blood bank specific ranges and averages should be established for the different blood groups if cryoprecipitate is used on a regular basis to facilitate the correct calculation of dosages. As an example, several authors found a higher content of factor VIII, mostly around 140 U/bag, than the estimated 100 U/bag commonly used in clinical practice (Table 19.2).

Attempts to prepare safer cryoprecipitate by treating it with methylene blue plus light resulted in considerable losses of factor VIII (27–40%) and fibrinogen (39–41%). It may, however be suitable for treatment of von Willebrand’s disease (vWD), as there was only small loss of vWF antigen and retention of a normal multimeric pattern. If treated with solvents and detergents as a step to inactivate viruses, the vWF antigen level was only 37 percent of control values with complete loss of highest molecular weight molecules. Consequently, solvent-detergent treated

**Table 19.1: Constituents of cryoprecipitate**

- Factor VIII
- Fibrinogen
- von Willebrand’s factor (vWF)
- Factor XIII
- Fibronectin
- Isohemagglutinins anti-A and anti-B
Use of Cryoprecipitate

211

cryoprecipitate is unsuitable as a replacement therapy for patients with vWD.10

Some blood centers offer single-donor cryoprecipitate derived from large volumes of plasma obtained by plasmapheresis. Donors can donate repeatedly for a designated recipient and exposure to multiple donors and the risk of transfusion-acquired infection in the recipient is reduced. To increase the level of vWF or factor VIII in the donor, they can be given desmopressin (DDAVP, 1-deamino-8-D-arginine vasopressin) immediately prior to each donation. This increases the yield in the resultant cryoprecipitate and donor exposure can be drastically lowered in some patients with hemophilia A or severe vWD.11,12

INDICATIONS FOR USE

Hypofibrinogenemia

Acquired hypofibrinogenemia can occur secondary to increased turnover in disseminated intravascular coagulation (DIC), obstetric complications, and systemic reactive or therapeutic hyperfibrinolytic states. Massive blood loss and severe liver disease are other causes for low fibrinogen. Congenital afibrinogenemia is a rare autosomal recessive condition with mild-to-severe bleeding tendency,13 whereas congenital dysfibrinogenemias are a heterogeneous group of disorders with most patients being asymptomatic while others can either suffer from bleeding tendency or thromboembolism, or occasionally both.14

Replacement of fibrinogen is indicated in patients with fibrinogen levels below 80 to 100 mg/dl and if recent or active bleeding is present15 or if needed prophylactically prior to surgery or during massive transfusion. Spontaneous hemorrhage does not usually occur if fibrinogen is at least 50 mg/dl, and levels above 100 mg/dl are generally considered adequate for hemostasis after surgery or trauma.16 Keeping fibrinogen above 150 mg/dl may be acceptable in newborns with significant risk for intracranial hemorrhage. In some countries, virus-inactivated fibrinogen concentrates are available and should be preferred to cryoprecipitate.

von Willibrand’s Disease

von Willibrand’s disease (vWD) is the most common inherited bleeding disorder. It is characterized by a quantitative or qualitative abnormality of vWF, a family of multimeric plasma proteins that mediate platelet adhesion to damaged endothelium and form complexes with factor VIII, which is important to maintain normal plasma levels of factor VIII. With more severe disease, the bleeding time and PTT are prolonged due to low factor VIII levels but with normal platelet counts. Diagnostic evaluation includes measurement of factor VIII:C (coagulant activity), vWF antigen (vWF:Ag), vWF activity (ristocetin cofactor activity, vWF:RCoF), and multimeric analysis of vWF.

There are many types of vWD. Type 1 vWD is the most common variant (70–80%) characterized by a partial deficiency of vWF.17 The bleeding tendency is

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Average amount*</th>
<th>Dosage guide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>250 mg/bag</td>
<td>To calculate dose:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Plasma volume in deciliters × (desired fibrinogen level in mg/dl – initial fibrinogen level in mg/dl) = mg of fibrinogen required.</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>100 U/bag</td>
<td>2. Bags of cryoprecipitate increase fibrinogen level by 7-10 mg/dl in 70 kg adult or an increase of more than 100 mg/dl in newborn</td>
</tr>
<tr>
<td>VWF</td>
<td>100 U/bag</td>
<td>Give one bag per 10 kg every 12 to 24 hours (major bleeding or surgery)</td>
</tr>
<tr>
<td>Factor XIII</td>
<td></td>
<td>1 bag per 10 to 20 kg every 3–4 weeks (maintenance therapy)22</td>
</tr>
</tbody>
</table>

* The average amount/bag may not be representative for every institution so that the number of bags may have to be adjusted accordingly
variable and depends on the levels of vWF and factor VIII.

Type 2 has 4 subtypes (2A, 2B, 2M, and 2N) and refers to qualitative abnormalities of vWF. Type 3 is the most severe form with complete lack of vWF and extremely low (2–10%) factor VIII levels. The clinical course resembles hemophilia A or B. The platelet-type vWD (pseudo-vWD) is actually a platelet dysfunction with increased binding of defect platelets to vWF and mild thrombocytopenia. Though it resembles Type 2B vWD, administration of vWF may aggrivate thrombocytopenia, whereas platelet transfusions are of therapeutical value.

Treatment depends on the type of disease and efforts have to be made to clarify the patient’s type and subtype to ensure optimal therapy. DDAVP, which stimulates the release of vWF from endothelial cells, is the mainstay of therapy in type 1. It is, however, usually ineffective in type 2A and contraindicated in type 2B, because the abnormal vWF can cause platelet aggregates resulting in thrombocytopenia following DDAVP use. Type 3 is usually unresponsive to DDAVP due to absent endothelial stores. Other drug treatment options are the use of estrogens in women and antifibrinolytic agents such as epsilon aminocaproic acid or tranexamic acid for minor mucosal membrane bleeding or as adjuncts during surgery, especially dental procedures. Strict avoidance of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) should be kept in mind. If DDAVP is ineffective or contraindicated, vWF replacement therapy is needed to manage bleeding episodes or as prophylaxis prior to surgery. Cryoprecipitate contains a sufficient amount of vWF in a concentrated form for management of such situations and is usually available. Repeated infusions every 12 to 24 hours are often needed with major bleeding episodes or major surgery. For the typical cryoprecipitate dose, see Table 19.2. If the commercial factor VIII concentrates rich in vWF or pure vWF concentrates treated by virucidal methods are available, they should be preferred to cryoprecipitate due to their reduced risk of transmitting donor-derived infections. Even though the bleeding time is not always corrected by these concentrates, they are clinically as effective as cryoprecipitate and perceived as safer. In rare cases, transfusion of platelet concentrates may be needed to stop bleeding in type 3 vWD.

**Factor VIII Deficiency (Hemophilia A)**

Hemophilia A is the X-chromosome-linked deficiency of factor VIII with the severity of the bleeding tendency dependent on factor VIII levels (mild disease: 6–30%, moderate: 1–5%, severe: less than 1%). Mild-to-moderate disease can often be managed without factor VIII replacement. DDAVP may raise factor VIII in mild hemophilias and antifibrinolytics may sometimes be helpful in minor bleeding episodes, especially related to dental procedures. A number of commercial recombinant or plasma-derived virucidally-treated factor VIII concentrates are available. Locally prepared cryoprecipitate should only be used for urgent treatment if other safer factor VIII concentrates are not available and if no factor VIII inhibitor is present in the patient. For dose calculation, see Table 19.2.

**Factor XIII Deficiency**

Deficiency of factor XIII (fibrin-stabilizing factor) is a rare autosomal recessive bleeding disorder typically causing umbilical bleeding and immediate or delayed bleeding after surgery or trauma, abnormal wound healing and high frequency of intracranial hemorrhage. Because factor XIII deficiency does not cause abnormal coagulation tests, a 5 M urea solubility test of the patient’s fibrin clot or a quantitative factor XIII test is required. The long in vivo half-life of factor XIII (11–14 days) and risk of bleeding in deficient patients warrants prophylactic treatment with factor XIII concentrate or, if unavailable, either FFP or cryoprecipitate (for dose, see Table 19.2). Factor XIII levels of 3 to 5 percent are sufficient in human plasma for normal hemostasis. For surgery, factor XIII level should be kept at 25 to 50 percent.

The role of factor XIII replacement in acquired deficiency states is still unclear. It may be beneficial if prolonged bleeding occurs in patients undergoing cardiovascular surgery or neurosurgery.

**Fibrin Sealant**

Topical use of fibrinogen along with thrombin as fibrin surgical adhesive (‘fibrin glue’) can serve as a useful tool to control local bleeding and thus reduce or avoid transfusions during surgical procedures. Various commercial products that have been subjected to virus-inactivation steps are available in many
countries. Cryoprecipitate can also be used as source of fibrinogen and can be dispensed locally by the blood bank. Fibrinogen and thrombin can be combined during surgery to produce local hemostasis. Autologous preparations are a possible option to avoid infectious disease transmission. Fibrin sealants are used in a wide range of surgical procedures including cardiothoracic, ENT, gastrointestinal, vascular, and neurosurgery. The adhesive and hemostatic properties of fibrin glue vary with the final composition of the product. Stronger clots are achieved with higher fibrinogen concentrations; whereas higher thrombin concentrations lead to more rapid clot formation, which is advantageous when suturing blood vessels, but less desirable for careful tissue adjustment. Supplementation by factor XIII may increase the stability of the clot and addition of an antifibrinolytic agent may be useful if applied to an area with high fibrinolytic activity. Side effects include the potential risk of disease transmission as well as the development of antibodies to thrombin and factor V, a very serious condition caused by the use of bovine thrombin.

**Uremic Bleeding**

Patients with chronic kidney disease frequently suffer from a defect in platelet function that correlates with the severity of uremia as well as the degree of anemia, and which prolongs bleeding time. Prolonged bleeding from puncture sites, subdural hematomas as well as nasal, gastrointestinal and genitourinary hemorrhage are among the most common manifestations. Dialysis, treatment of anemia with erythropoietin, treatment with DDAVP and conjugated estrogens are all effective treatment options in uremic platelet dysfunction. Infusion of cryoprecipitate to treat bleeding episodes or prior to surgery is only indicated if other options, especially hemodialysis and administration of DDAVP, have failed, are contraindicated, or not available.

**Reversal of Fibrinolytic Therapy**

If bleeding occurs during fibrinolytic therapy, it can usually be controlled by stopping the infusion since the half-life of most fibrinolytic agents is short (5–30 minutes). In cases of severe bleeding, particularly if immediate surgery is required, reversal of fibrinolysis may be necessary. This can be achieved by administration of antifibrinolytic drugs such as epsilon aminocaproic acid or tranexamic acid. Infusion of cryoprecipitate may be necessary for fibrinogen repletion.

**ADMINISTRATION AND DOSAGE**

Prior to infusion cryoprecipitate is rapidly thawed at 30° to 37°C in a water bath. To prevent contamination of the bags by unsterile water, a plastic overwrap or a device that keeps the ports out of the water needs to be used. Several bags may be pooled into one container either prior to transfusion or before freezing. Thawed cryoprecipitate is stored at room temperature. It must be transfused within 4 hours of first entry if it was pooled and if not pooled within 6 hours after thawing. Fibrinogen repletion.33

**REFERENCES**


Coagulation Therapy in Hemophilia

Mark T Reding

Although hemophilia was recognized centuries ago, successful treatment of this life-threatening coagulopathy has only become possible in the last few decades. Beginning with the discovery of cryoprecipitate in the mid-1960s, significant progress has been made with the development of safe and effective clotting factor replacement therapy. We are now on the verge of gene therapy for hemophilia, which may finally provide a true cure for this devastating disease. However, much work needs to be done before gene therapy is a clinical reality, leaving coagulation factor replacement therapy as the mainstay of hemophilia treatment today.

Hemophilia is an inherited bleeding disorder caused by deficiency of coagulation factor VIII (fVIII) or factor IX (fIX). This results in absence of the intrinsic factor X activating complex that is required for the burst of thrombin generation necessary to form and maintain a stable clot in the face of hemostatic challenges. Hemophilia A (fVIII deficiency) occurs in approximately 1 of every 5,000 to 10,000 male births. Hemophilia B (fIX deficiency) is less common, occurring in approximately 1 of every 30,000 male births. Hemophilia A and B occur in all racial and ethnic groups. The genes for fVIII and fIX are both located on the long arm of the X chromosome, and the inheritance pattern is thus sex linked. Approximately 30 percent of cases represent new spontaneous mutations; and, therefore, occur in the absence of any known family history of hemophilia.

Hemophilia A and B are clinically indistinguishable, although the severity of the disease varies. Individuals with fVIII or fIX levels of less than 1 percent are said to have severe hemophilia, which is characterized by frequent spontaneous bleeding into joints and soft tissues, and prolonged bleeding with even minor trauma or surgery. Recurrent hemarthroses and soft tissue hemorrhages may lead to chronic pain and disability from severe arthropathy, joint contractures, and pseudotumors. Those with 2 to 5 percent fVIII or fIX have moderate hemophilia, which is characterized by excessive bleeding with trauma or surgery but only occasional spontaneous bleeding. Mild hemophilia (greater than 5% fVIII or fIX activity) is characterized by bleeding only with significant trauma or surgery, lack of spontaneous bleeding, and unlike severe or moderate hemophilia, may not be recognized until adulthood.

EVOLUTION OF COAGULATION FACTOR REPLACEMENT THERAPY

Hemophilia A

The modern era of hemophilia therapy began in 1964 when Dr Judith Graham Pool and colleagues reported that the precipitate of fresh frozen plasma (FFP) (“cryoprecipitate”) contained high concentrations of fVIII. For the first time, fVIII levels approaching 30 percent (the approximate amount required for normal hemostasis) could be obtained. Dramatic increases in the life expectancy of those with hemophilia A soon followed. In the early 1970s, the process of pooling together the plasma from thousands of donors to produce high potency dried plasma concentrates of fVIII was developed. While this process revolutionized the treatment of hemophilia A by allowing for self-administration and home treatment, it also greatly enhanced the risk of transmitting blood-borne
infections such as viral hepatitis and HIV. Thus began the development of techniques to reduce and ultimately eliminate contamination of fVIII concentrates in the 1980s. Heat treatment of plasma-derived clotting factor concentrates was introduced in 1984. Improved donor screening and enhanced viral inactivation/elimination techniques such as pasteurization, solvent/detergent treatment, immunoaffinity chromatography, and gel filtration chromatography soon followed, beginning in the mid to late 1980s.7

Plasma-derived fVIII products are often classified based on their final purity, which is defined as international units (IU) of clotting factor activity per milligram of protein. Because they also contain other plasma proteins such as fibrinogen, fibronectin and other noncoagulant proteins, intermediate purity products have a relatively low specific activity (less than 50 IU/mg). Aside from albumin that acts as a stabilizer, high purity (more than 50 IU/mg) and ultra-high purity (more than 3,000 IU/mg) products contain little or no other contaminating plasma proteins.

Another major advance occurred in 1984 with the identification of the structure and cloning of fVIII.8,9 Together with the development of recombinant DNA technology, this lead to the introduction of the first generation recombinant fVIII products in the early 1990s. The first generation recombinant fVIII products all contain human albumin as a stabilizer. While they have an excellent safety record, theoretic concern about the potential for transmitting infectious agents remains. This has lead to the introduction of the second generation recombinant fVIII products in early 2000. These products contain sucrose as a stabilizer, yet human plasma proteins are still used in the cell culture process. The third generation of recombinant fVIII products are manufactured and formulated without the addition of any human or animal proteins. Tables 20.1 and 20.2 list currently available plasma derived and recombinant fVIII products.

Hemophilia B
Unlike fVIII, fIX is not present in hemostatically meaningful amounts in cryoprecipitate. During the 1970s and 1980s, the mainstay of fIX replacement therapy

<table>
<thead>
<tr>
<th>Type/Product name</th>
<th>Manufacturer</th>
<th>Stabilizer</th>
<th>Method of viral inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>First generation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioclate</td>
<td>Genetics Institute/Baxter</td>
<td>Albumin</td>
<td>IA, IE, filtration</td>
</tr>
<tr>
<td>Kogenate / Helixate</td>
<td>Bayer/ZLB Behring</td>
<td>Albumin</td>
<td>IA, IE, filtration</td>
</tr>
<tr>
<td>Recombinate</td>
<td>Baxter</td>
<td>Albumin</td>
<td>IA, IE, filtration</td>
</tr>
<tr>
<td>Second generation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kogenate FS/ Helixate FS</td>
<td>Bayer/ZLB Behring</td>
<td>Sucrose</td>
<td>IA, IE, S/D, filtration</td>
</tr>
<tr>
<td>Refacto*</td>
<td>Wyeth</td>
<td>Sucrose</td>
<td>IA, IE, S/D, filtration</td>
</tr>
<tr>
<td>Third generation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advate</td>
<td>Baxter</td>
<td>Trehalose</td>
<td>IA, IE, S/D, plasma free culture</td>
</tr>
</tbody>
</table>

IA = immunoaffinity chromatography
IE = ion exchange chromatography
S/D = solvent/detergent

*Note: Refacto is a B domain deleted fVIII product. Plasma fVIII activity levels measured with a standard one stage clotting assay can be up to 50% lower than expected, unless a Refacto Laboratory Standard is utilized. Alternatively, Refacto can be monitored with a chromogenic fVIII assay.
Coagulation Therapy in Hemophilia

for patients with hemophilia B consisted of concentrates prepared from plasma or supernatant of cryoprecipitate. These so-called fIX complex concentrates or prothrombin complex concentrates (PCC) also contain fX, fVII, and prothrombin. As for plasma-derived fVIII concentrates, the emergence of blood-borne infections led to modifications in the manufacturing process of PCCs to reduce potential viral contamination. In the late 1980s and early 1990s, high purity plasma-derived fIX concentrates became available. These high purity fIX concentrates have a potential advantage over PCCs in that they are less thrombogenic particularly in patients with hepatic insufficiency.

Although the cDNA for fIX was cloned and the fIX gene was sequenced in the mid-1980s, the first and only recombinant fIX product became commercially available almost a decade later. This recombinant fIX product is manufactured without the addition of any animal or human proteins. Tables 20.3 and 20.4 list currently available PCCs and high purity fIX concentrates.

**CLINICAL APPLICATION OF COAGULATION FACTOR REPLACEMENT THERAPY**

**Product Choice**

The selection of a particular clotting factor product to treat or prevent a bleeding episode is influenced by several variables including product cost and availability, as well as physician and patient preference. With the availability of plasma derived and recombinant clotting factor concentrates, the use of cryoprecipitate in patients with hemophilia A is generally a last resort. Similarly, the PCCs now play a larger role in the management of patients with fVIII inhibitors than in the treatment of patients with hemophilia B. The choice between plasma derived and recombinant factor products may be difficult, although cost and availability often determine the selection. While plasma-derived products are now far safer than in the past, a patient’s HIV and viral hepatitis status may also affect the decision-making process.

Several large clinical trials have evaluated the safety and efficacy of the various recombinant fVIII products in both previously untreated and previously treated patient populations. From a practical perspective, all plasma derived and recombinant fVIII products are considered to be therapeutically equivalent. The same holds true for the fIX products, with the caveats that the recovery of fIX activity levels following bolus infusion has more interpatient variability and the recovery of recombinant fIX is approximately 20 percent lower than for high purity plasma-derived fIX products. This variable recovery and lower than expected recovery of recombinant fIX is particularly an issue in infants and small children.

**Dosing and Duration of Therapy**

The dosing of coagulation factor replacement therapy is determined by several factors. The level of clotting factor activity necessary to achieve and maintain adequate hemostasis or prophylaxis, plasma volume and distribution of the clotting factor protein between intra- and extravascular spaces, the circulating half-life of the clotting factor, and the patient’s previous response to treatment all must be considered.

**Table 20.3: Prothrombin complex concentrates (plasma derived)**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Manufacturer</th>
<th>Method of viral inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bebulin</td>
<td>Baxter</td>
<td>Vapor heat</td>
</tr>
<tr>
<td>Konyne 80</td>
<td>Bayer</td>
<td>Dry heat</td>
</tr>
<tr>
<td>Profilnine SD</td>
<td>Grifols</td>
<td>Solvent/detergent</td>
</tr>
<tr>
<td>Proplex T</td>
<td>Baxter</td>
<td>Dry heat</td>
</tr>
<tr>
<td>Autoplex T*</td>
<td>Baxter</td>
<td>Dry heat</td>
</tr>
<tr>
<td>Feiba VH*</td>
<td>Baxter</td>
<td>Vapor heat</td>
</tr>
</tbody>
</table>

*Contain variable amounts of activated clotting factors (often referred to as “activated PCCs”)

**Table 20.4: High purity factor IX concentrates**

<table>
<thead>
<tr>
<th>Type/Product name</th>
<th>Manufacturer</th>
<th>Method of viral inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma derived</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alphanine SD</td>
<td>Grifols</td>
<td>IA, S/D, filtration</td>
</tr>
<tr>
<td>Mononine</td>
<td>ZLB Behring</td>
<td>IA, chemical treatment, filtration</td>
</tr>
<tr>
<td>Recombinant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benefix*</td>
<td>Wyeth</td>
<td>IA, filtration, plasma free culture</td>
</tr>
</tbody>
</table>

S/D = solvent/detergent  
IA = immunoaffinity chromatography  
*Note: The recovery of plasma fIX activity with Benefix is approximately 20% lower than for plasma-derived high purity fIX concentrates. See text for details.
general, 1 IU fVIII/kg body weight will increase the plasma fVIII level by 0.02 IU/ml (2%). Because of a larger volume of distribution, 1 IU fIX/kg body weight will only increase the plasma fIX level by 0.01 IU/ml (1%). Typical recovery of plasma fIX activity with recombinant fIX is approximately 20 percent lower than for plasma-derived fIX concentrates, and thus, a dose of 1.2 IU recombinant fIX/kg body weight is generally required to increase the plasma fIX level by 1 percent.

For life- or limb-threatening hemorrhage, serious trauma, or major surgical procedures, the plasma level of clotting factor actually achieved should be verified by direct measurement. In the setting of active bleeding or major surgery, increased consumption and/or enhanced clearance of clotting factors by non-neutralizing antibodies may necessitate additional dosing to achieve the desired factor level. Life- or limb-threatening hemorrhage, serious trauma, and major surgery all require that a minimum of 80 to 100 percent of normal clotting factor activity be maintained. A minimum activity level of 50 percent of normal should be maintained for moderate-to-severe bleeding into joints or soft tissues. For minor bleeding or surgical procedures, factor activity levels of 30 percent may suffice. It is important to note that patients with hemophilia B may often be treated adequately with target factor levels that are up to 25 percent lower than target levels for patients with hemophilia A. This empiric experience is reflected in the treatment guideline recommendations listed in Tables 20.5 and 20.6. It is important to emphasize that these observations are based largely on empiric experience gained over many years and specific recommendations for each patient should be individualized and based on clinical experience and response to bleeding episodes.

Frequency of dosing is determined by the half-life of the clotting factor being replaced. The half-lives of fVIII and fIX are 8 to 12 hours and approximately 18 hours, respectively. Many centers in the United States use continuous infusion regimens rather than intermittent bolus injections, particularly in the post-operative setting and in the case of bleeding severe enough to require hospitalization. Continuous infusion maintains a stable therapeutic level of clotting factor activity, avoids the “peak and trough” effect, and allows for ease of laboratory monitoring with random blood samples. In patients with hemophilia A, a continuous infusion of 4 to 6 IU fVIII/kg/hr should be started immediately following an initial bolus dose. In those with hemophilia B, a continuous infusion of 3 to 4 IU fIX/kg/hr may be used. In either case, monitoring of factor levels is essential.

The duration of coagulation factor replacement therapy is largely empiric and should be individualized. Some minor invasive or surgical procedures only require a single prophylactic dose, while others may require up to 7 days of prophylactic treatment. Most hemarthroses can be successfully treated with 1 to 3 doses of clotting factor, depending on how quickly the symptoms are recognized and the patient’s response to treatment. For major surgery or episodes of life- or limb-threatening hemorrhage, treatment for 7 to 14 days (or perhaps longer) is usually indicated.

Tables 20.5 and 20.6 summarize treatment guidelines for coagulation factor replacement therapy utilized at our institution. They are typical of those used at other hemophilia treatment centers in the United States. It is recognized, however, that clinical practice in other countries (particularly those with limited access to coagulation factor concentrates) often employs doses lower than those shown, and still achieves satisfactory results.

Prophylactic Coagulation Factor Replacement Therapy

With the improved safety of plasma-derived clotting factor concentrates and the availability of recombinant products, prophylactic factor replacement therapy has become more common. Primary prophylaxis involves the regular (2–3 times/wk) and long-term administration of clotting factor, beginning in early childhood (1–2 years of age) with the goal of preventing hemarthroses and subsequent joint disease. Secondary prophylaxis is the regular use of clotting factor, generally over a period of 3 to 6 months, to reduce the amount of bleeding in a target joint with the goal of decreasing the progression of joint disease. While several studies have shown clear benefit from primary prophylaxis, weaknesses of these studies include their retrospective design and lack of uniform defi-
### Table 20.5: General guidelines for coagulation factor replacement therapy

<table>
<thead>
<tr>
<th>Type of bleeding event</th>
<th>Hemophilia A (factor VIII dose)</th>
<th>Hemophilia B (factor IX dose)</th>
<th>Special considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected intracranial hemorrhage</td>
<td>100 IU/kg bolus, followed by 4–6 IU/kg/hr continuous infusion</td>
<td>150 IU/kg bolus, followed by 3-4 IU/kg/hr continuous infusion</td>
<td>Keep factor level &gt;80%</td>
</tr>
<tr>
<td>Life-threatening hemorrhage (injury or bleeding in the head, neck, chest, or abdomen; GI bleeding; major surgery)</td>
<td>50 IU/kg, followed by 30 IU/kg every 8 hours</td>
<td>100 IU/kg, followed by 40 IU/kg every 12–24 hours</td>
<td>Continuous infusion may also be used in place of repeat bolus doses. Keep factor level &gt;80%.</td>
</tr>
<tr>
<td>Severe hemorrhage into joints or soft tissues</td>
<td>40–50 IU/kg, followed by 20–30 IU/kg every 8–12 hours</td>
<td>50–100 IU/kg, followed by 30 IU/kg every 12–24 hours</td>
<td>Rest/immobilization, ice, and elevation are useful adjuncts</td>
</tr>
<tr>
<td>Minor hemorrhage into joints or soft tissues</td>
<td>20–30 IU/kg, repeat every 12 hours</td>
<td>20–40 IU/kg, repeat every 24 hours</td>
<td>Follow-up dose may not be necessary</td>
</tr>
<tr>
<td>Gross hematuria</td>
<td>30–40 IU/kg, repeat every 12 hours</td>
<td>30–50 IU/kg, repeat every 24 hours</td>
<td>Bedrest, force fluids. Consider prednisone 20 mg po tid × 5 days</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>15 IU/kg</td>
<td>20 IU/kg</td>
<td>Use of antifibrinolytic agent for 3 days is often helpful. May need to extend to 10 days for recurrent epistaxis.</td>
</tr>
</tbody>
</table>

**Notes:**
1. These are standardized guidelines used in our institution. Specific dosing recommendations should be individualized based on clinical circumstances and the discretion of the treating physician.
2. FVIII doses listed are for plasma derived or recombinant products.
3. FIX doses listed are for recombinant fIX (used most commonly in our institution). Optimal doses for plasma-derived fIX products may be slightly different—see text for details.
4. Typical recovery of recombinant fIX is lower in pediatric patients than in adults, and higher doses should be considered.

### Table 20.6: Prophylactic coagulation factor replacement guidelines for specific procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Hemophilia A (factor VIII dose)</th>
<th>Hemophilia B (factor IX dose)</th>
<th>Hemophilia B Pediatric, &lt; 40 kg (factor IX dose)</th>
<th>Special considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple extractions</td>
<td>30 IU/kg</td>
<td>20 IU/kg</td>
<td>40 IU/kg</td>
<td>Antifibrinolytic agent should also be used for 7–14 days. Avoid mandibular nerve block.</td>
</tr>
<tr>
<td>Complex extractions, gingival surgery</td>
<td>50 IU/kg, followed by 30 IU/kg every 24 hr × 3 days</td>
<td>60 IU/kg, followed by 20 IU/kg every 24 hr × 3 days</td>
<td>80 IU/kg, followed by 40 IU/kg every 24 hr × 3 days</td>
<td></td>
</tr>
<tr>
<td>Fillings with local anesthetic infiltration</td>
<td>25 IU/kg</td>
<td>40 IU/kg</td>
<td>60 IU/kg</td>
<td></td>
</tr>
<tr>
<td>Routine cleaning, orthodontics</td>
<td></td>
<td></td>
<td></td>
<td>No treatment necessary</td>
</tr>
<tr>
<td>Central venous catheter Placment</td>
<td>50 IU/kg, followed by 30 IU/kg every</td>
<td>60 IU/kg, followed</td>
<td>80 IU/kg, followed</td>
<td></td>
</tr>
</tbody>
</table>

Contd...
### Table: Hemophilia Management

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Hemophilia A (factor VIII dose)</th>
<th>Hemophilia B Adults (factor IX dose)</th>
<th>Hemophilia B Pediatric, &lt; 40 kg (factor IX dose)</th>
<th>Special considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal</td>
<td>12 hr × 3 days, then every 24 hr × 4 days</td>
<td>by 20 IU/kg every 24 hr × 7 days</td>
<td>by 40 IU/kg every 24 hr × 7 days</td>
<td></td>
</tr>
<tr>
<td>Removal</td>
<td>30 IU/kg, followed by 20 IU/kg every 24 hr × 2 days</td>
<td>20 IU/kg, followed by 20 IU/kg every 24 hr × 2 days</td>
<td>40 IU/kg, followed by 40 IU/kg every 24 hr × 2 days</td>
<td>Do not access Port-A-Cath immediately following placement. Use peripheral IV for follow-up factor infusions for 7 days</td>
</tr>
<tr>
<td>Port-A-Cath Placement</td>
<td>50 IU/kg, followed by 30 IU/kg every 8 hr × 1 day, then 30 IU/kg every 24 hr × 3 days</td>
<td>60 IU/kg, followed by 20 IU/kg every 12 hr × 3 days, then 30 IU/kg every 24 hr × 4 days</td>
<td>80 IU/kg, followed by 40 IU/kg every 12 hr × 3 days, then 60 IU/kg every 24 hr × 4 days</td>
<td></td>
</tr>
<tr>
<td>Removal</td>
<td>50 IU/kg, followed by 30 IU/kg every 12 hr × 1 day, then 30 IU/kg every 24 hr × 2 days</td>
<td>60 IU/kg, followed by 20 IU/kg every 12 hr × 1 day, then 30 IU/kg every 24 hr × 2 days</td>
<td>80 IU/kg, followed by 40 IU/kg every 12 hr × 2 days</td>
<td></td>
</tr>
<tr>
<td>Bronchoscopy/Endoscopy Without biopsy</td>
<td>30 IU/kg</td>
<td>20 IU/kg</td>
<td>40 IU/kg</td>
<td></td>
</tr>
<tr>
<td>Bronchoscopy/Endoscopy With biopsy</td>
<td>50 IU/kg, followed by 30 IU/kg every 12 hr × 3 days</td>
<td>60 IU/kg, followed by 20 IU/kg every 24 hr × 3 days</td>
<td>80 IU/kg, followed by 40 IU/kg every 24 hr × 3 days</td>
<td></td>
</tr>
<tr>
<td>Lumbar puncture</td>
<td>50 IU/kg</td>
<td>40 IU/kg</td>
<td>60 IU/kg</td>
<td></td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
<td>50 IU/kg, followed by 30 IU/kg every 24 hr × 2 days</td>
<td>60 IU/kg, followed by 30 IU/kg every 24 hr × 2 days</td>
<td>80 IU/kg, followed by 60 IU/kg every 24 hr × 2 days</td>
<td></td>
</tr>
<tr>
<td>Joint aspiration/ injection</td>
<td>40 IU/kg, followed by 30 IU/kg every 12 hr × 2 days</td>
<td>30 IU/kg, followed by 20 IU/kg every 24 hr × 2 days</td>
<td>60 IU/kg, followed by 40 IU/kg every 24 hr × 2 days</td>
<td>Immobilization also advised</td>
</tr>
<tr>
<td>Aggressive physical therapy</td>
<td>30 IU/kg</td>
<td>20 IU/kg</td>
<td>40 IU/kg</td>
<td>Daily, prior to physical therapy</td>
</tr>
<tr>
<td>Arterial puncture</td>
<td>30 IU/kg, repeat dose in 12 hr</td>
<td>20 IU/kg, repeat dose in 12 hr</td>
<td>40 IU/kg, repeat dose in 12 hr</td>
<td>Direct pressure × 10 minutes. Apply pressure dressing.</td>
</tr>
<tr>
<td>Intramuscular injection</td>
<td>No treatment necessary</td>
<td></td>
<td></td>
<td>Use small gauge needle. Ice site for 5–10 minutes. Apply pressure dressing.</td>
</tr>
</tbody>
</table>

**Notes:**

1. These are standardized guidelines used in our institution. Specific dosing recommendations should be individualized based on clinical circumstances and the discretion of the treating physician.
2. FVIII doses listed are for plasma derived or recombinant products.
3. FIX doses listed are for recombinant fIX (used most commonly in our institution). Optimal doses for plasma-derived fIX products may be slightly different—see text for details.
4. Typical recovery of recombinant fIX is lower in pediatric patients than in adults, and higher doses are thus indicated.
Coagulation Therapy in Hemophilia

Complications of Prophylaxis

The results of an ongoing study designed to compare primary prophylaxis vs. aggressive on-demand treatment are eagerly anticipated. The aim of prophylaxis is to maintain trough factor activity levels of 1 to 3 percent. In severe hemophilia A, this is typically achieved by doses of approximately 30 IU fVIII/kg given three times per week. For hemophilia B, doses of 20 to 40 IU fIX/kg are usually given twice per week.

Complications of Coagulation Factor Replacement Therapy

Despite the significant advances in reducing the likelihood of transmitting infectious diseases, coagulation factor replacement therapy still has risks. Infection remains perhaps the most feared risk, although complications associated with central venous access devices (CVADs), allergic reactions, and the development of inhibitors are all far more likely to occur.

Viral hepatitis was a recognized and accepted complication of treatment with clotting factor concentrates until the AIDS epidemic in the early 1980s forever changed our perception of the true magnitude of the threat of viral transmission and its potentially devastating consequences. During that time, up to 70 percent of patients with severe hemophilia who had been treated with plasma-derived concentrates in the United States and Europe became infected with HIV. This tragedy led to the rapid development of the improved viral elimination techniques that render current clotting factor concentrates virtually free of all known viral pathogens. However, hepatitis C virus is still a major cause of chronic liver disease and death in the hemophilia population, given that up to 90 percent of those who received older plasma-derived concentrates became infected. Current purification and viral inactivation methods dramatically reduced both the total number of deaths and HIV-related deaths in the hemophilia population in the mid to late 1990s. More importantly, only 37 cases of HIV seroconversion were identified in hemophilia patients between 1987 and 1990, and none of them had received currently available plasma derived or recombinant factor products. Since 1990, no documented cases of HIV seroconversion have been attributed to the products currently in clinical use.

Nonetheless, concern remains about the potential for transmission of as yet undiscovered viruses, prions, and other infectious agents. The third generation recombinant fVIII products and recombinant fIX, which are manufactured without exposure to any human or animal proteins (although a murine monoclonal antibody is used in the purification process for Advate) offer the best reassurance against this theoretical risk.

The need to administer frequent infusions of clotting factor concentrates, particularly for prophylaxis, often necessitates the placement of a CVAD. The main complication of CVAD use is bacterial infection, which occurs in 30 percent or more of patients, often within the first few months of use. Furthermore, thrombotic occlusion of the central veins is being increasingly reported, particularly in children. Although many are asymptomatic, venographic evidence of thrombosis is seen in up to 50 percent of children after 5 years of CVAD use. The longer term consequences of this are not yet known.

As with any infused protein, the use of coagulation factor products may cause allergic reactions. Although infrequent, symptoms such as rash, pruritus, fever, tachycardia, tachypnea, abdominal pain, or nausea may occur. Life-threatening symptoms of anaphylaxis (bronchospasm, angioedema, hypotension) may also occur, particularly in patients with severe hemophilia B. The incidence of this type of severe allergic reaction is approximately 5 percent and may be precipitated by any fIX containing product. Patients who go on to develop fIX inhibitors seem to be particularly at risk for these severe allergic reactions. Although premedication with acetaminophen, antihistamines, and corticosteroids may allow for the continued use of coagulation factor products in patients who experience allergic reactions, a history of anaphylaxis is obviously a strict contraindication to any further use of the offending product.

The development of antibodies which neutralize the procoagulant function of fVIII or fIX (inhibitors) is a severe complication of treatment with coagulation factor products and will be further considered below.

Desmopressin

Desmopressin (1-deamino-8-D-arginine vasopressin, or DDAVP) is a synthetic analogue of the antiuretic
hormone arginine vasopressin. Intravenous, subcutaneous, or intranasal administration of DDAVP results in transient increases in plasma concentrations of fVIII and vWF due to their release from vascular endothelium. Peak levels (typically 2–4 times basal) are achieved 30 to 60 minutes after intravenous and 60 to 90 minutes after subcutaneous or intranasal administration. Expression of glycoprotein (GP) Ib and GPIIb/IIIa on platelet membranes is also enhanced following administration of DDAVP.

DDAVP plays an important role in the management of mild hemophilia A. It may be given in advance of dental work or minor surgical procedures, following spontaneous bleeding events or trauma, and may help avoid the need for factor replacement therapy. The standard dose is 0.3 mcg/kg given in 50 cc normal saline over 30 to 60 minutes. Doses may be repeated at intervals of 12 to 24 hours, but tachyphylaxis may occur after 3 or 4 doses, limiting further usefulness of DDAVP. Intranasal DDAVP (Stimate, ZLB Behring) is given at a dose of 1 spray (150 mcg) in each nostril (total dose 300 mcg) for those weighing more than 50 kg. For patients less than 50 kg, the dose is 1 spray (150 mcg) in one nostril (total dose 150 mcg).

Common side effects include mild facial flushing and headache; water retention and hyponatremia may also occur. Fluid intake should thus be restricted for 24 hours following treatment with DDAVP, particularly in children. Myocardial infarction following the use of DDAVP has been reported; it should thus be used with caution in patients with known or suspected coronary artery disease, those with cardiac risk factors, and it is contraindicated in those with unstable coronary artery disease. Meta-analysis has also shown a 2.4-fold increase in perioperative myocardial infarction in cardiac surgery patients treated with DDAVP, and its routine use is not recommended in that setting.

### Antifibrinolytic Agents

Antifibrinolytic agents are often overlooked yet potentially useful ancillary treatment for those with both hemophilia A and B. By inhibiting plasmin-mediated clot lysis, these agents can help maintain the integrity of the clot, thus preventing delayed bleeding and reducing the need for further factor replacement therapy. Two antifibrinolytic agents are commercially available: epsilon aminocaproic acid (Amicar, Xanodyne Pharmaceuticals) and tranexamic acid (Cyklokapron, Pharmacia and Upjohn), although the latter is no longer available in the United States. Both can be administered orally, intravenously, or topically and are particularly useful in the management of mucous membrane bleeding from the oropharynx, nose, and lower genitourinary tract given that these sites secrete natural fibrinolytic enzymes. Their use should be avoided, however, in the setting of known or possible upper genitourinary tract bleeding due to the risk of thrombotic occlusion of the ureters. Their use is also contraindicated in those receiving PCCs or in whom there is evidence of disseminated intravascular coagulation (DIC).

Optimal dosing and duration of therapy are not well defined, but Amicar is typically dosed at 50 to 70 mg/kg orally every 6 to 8 hours (not to exceed 24 grams in 24 hours) and given for 3 to 14 days, while Cyklokapron is given at a dose of 25 mg/kg orally every 6 to 8 hours for 2 to 8 days.

### MANAGEMENT OF PATIENTS WITH INHIBITORS

The development antibodies that neutralize the procoagulant activity of fVIII or fIX is arguably the most serious complication of coagulation factor replacement therapy. Inhibitors remain a relatively common complication, affecting 25 to 35 percent of patients with severe hemophilia A. FVIII inhibitors may also develop in individuals without congenital fVIII deficiency and cause “acquired hemophilia.” This rare coagulopathy (incidence of approximately 0.2–1.0 per million of the general population per year) is associated with significant morbidity and a mortality rate of approximately 20 percent. A complex interplay of several factors including the type of fVIII mutation, HLA type, individual immunologic response characteristics, frequency and pattern of fVIII exposures, and product type may all play a role in the development of an inhibitor in a given hemophilia A patient. In addition, the frequency of screening and the sensitivity of the antibody detection methods used may also affect the perceived incidence of fVIII inhibitor formation. Acquired hemophilia represents a failure of the tolerance mechanisms that regulate normal immune responses to fVIII. Acquired fVIII inhibitors may be seen in association with collagen-vascular and other autoimmune diseases, solid tumors and lymphoproliferative disorders, pregnancy, and...
The development of an inhibitor should be suspected whenever active bleeding fails to subside or a patient does not respond as anticipated to treatment with clotting factor concentrates given in doses that would be expected to increase the plasma factor activity to levels sufficient for hemostasis. Inhibitors are occasionally detected by routine screening, in the absence of any clinical suspicion, although these are generally present in low amounts. FVIII inhibitors are usually quantified using the Bethesda assay. One Bethesda Unit is the amount of inhibitory activity that produces 50 percent inhibition of FVIII activity in a one-stage clotting assay. Patients are defined as having “low titer” inhibitors if the titer is less than 10 BU/ml, and “high titer” inhibitors if the titer is greater than 10 BU/ml. This distinction has important implications for clinical management, which consists of two broad aspects: (i) treatment of acute hemorrhage, and (ii) suppression of inhibitor production.

**Treatment of Acute Hemorrhage**

The management of an acutely bleeding inhibitor patient should take into consideration the current inhibitor titer and the patient’s previous response to bypassing agents. “Low responders” (those with an inhibitor titer of 10 BU/ml or less and no history of anamnestic rise in titer after exposure to FVIII) may often be successfully treated with higher doses of plasma derived or recombinant FVIII. “High responders” whose inhibitor titers are low at the time of a bleeding episode may also be initially treated with high dose FVIII, but inhibitor titers will rise quickly after approximately five days, making further treatment with FVIII alone futile. The infusion of high dose FVIII is usually preferable in patients with low inhibitor titers who experience life- or limb-threatening hemorrhage. In patients with higher inhibitor titers, the use of plasma exchange or extracorporeal immunoadsorption to physically remove the inhibitor antibodies can facilitate the use of high-dose FVIII infusion. The most efficient devices employ the use of columns containing either staphylococcal protein A or polyclonal sheep antibodies against human FVIII.

Porcine FVIII (plasma derived) has been used successfully for a number of years to treat inhibitor patients with both congenital and acquired hemophilia. Approximately 25 percent of patients with congenital hemophilia will have inhibitor antibodies which do not crossreact with porcine FVIII. While crossreactivity also occurs in those with acquired inhibitors, the antibody titer against porcine FVIII is often low enough to be clinically irrelevant. Testing to establish baseline titers against both human and porcine FVIII in patients who develop inhibitors can thus identify those for whom porcine FVIII may be a therapeutic option. Unfortunately, porcine FVIII has recently become unavailable, but early phase clinical trials of a recombinant porcine FVIII are underway.

Another therapeutic approach is to attempt to “bypass” the FVIII inhibitor with agents that induce hemostasis in the absence of FVIII or FIX. Activated prothrombin complex concentrates (aPCCs) have been used for this purpose since the early 1980s, and recombinant FVIIa (NovoSeven, Novo Nordisk) was first approved in Europe in 1996. Both agents provide activated coagulation factors that enhance thrombin generation on the surface of activated platelets, although the exact mechanisms of action are not fully understood. The therapeutic efficacy of aPCCs is reported to be in the 50 to 90 percent range, and disadvantages include the inability to monitor for hemostatic effect in vitro, unpredictable response, and potential for thrombotic complications. They may also contain trace amounts of FVIII which can induce an anamnestic antibody response.

There is ample experience from clinical trials in hemophilia patients undergoing surgery, home therapy for bleeding episodes, and a large compassionate use study documenting the usefulness of FVIIa in the management of inhibitors. While the recommended dose is 90 to 120 micrograms/kg infused every 2 to 3 hours, some patients require much higher initial doses (200–300 mcg/kg) and the minimal effective dose and optimal dose schedule are not yet clearly defined. Clinical trials are currently underway to directly compare standard vs. high dose FVIIa. The time between onset of bleeding symptoms and initiation of therapy appears to have a significant impact on the efficacy of FVIIa. Like aPCCs, there is no reliable laboratory test to monitor hemostatic
efficacy of rtVIIa, and ongoing treatment decisions must be made largely based on clinical judgment. Thrombosis is still a potential risk, although with an incidence of less than 1 percent, rtVIIa is much safer in this regard. The main disadvantage is extremely high cost, which may limit availability. A prospective randomized trial directly comparing aPCC and rtVIIa has not yet been published, although at two such studies are underway.

**Suppression of Inhibitors**

A goal in the management of any patient unfortunate enough to develop a fVIII inhibitor should be the long-term suppression of inhibitor production. In this regard, treatment strategies differ for patients with congenital hemophilia and patients with acquired inhibitors.

Immune tolerance induction (ITI) therapy was developed in the 1970s, and involves the regular (3–7 days per week) administration of relatively large doses of fVIII over an extended period of time (many months). Several different regimens have been developed, and debate persists over the optimal method to achieve successful immune tolerance. Some regimens include the use of adjunctive immunomodulation with cyclophosphamide or corticosteroids, although there is increasing reluctance to use these agents routinely, particularly in children. Overall success rates of approximately 70 percent have been reported by the International Immune Tolerance Registry (IITR), North American Immune Tolerance Registry (NAITR), German, and Spanish registries. A low inhibitor titer at the initiation of ITI (less than 10 BU/ml) and a low historical peak titer (less than 200 BU/ml) are predictors of success. Young age at the initiation of ITI and a shorter interval of time between inhibitor diagnosis and initiation of ITI may also predict a favorable outcome. The most controversial issue regarding ITI is the dose of fVIII required to successfully achieve immune tolerance. The International Immune Tolerance Study was launched in 2002 and will compare low dose fVIII (50 IU/kg three times per week) with high dose fVIII (200 IU/kg daily). This study will hopefully address some of the important unresolved issues surrounding the use of ITI. Continued prophylactic use of fVIII is commonly done after successful ITI, although whether this is required to maintain tolerance is unknown. The probability of eliminating inhibitors that persist after 2 years of ITI is extremely small, but the relapse rate after successful ITI is less than 5 percent.

Although of uncertain efficacy in inhibitor patients with congenital hemophilia, immunosuppression has been widely used in the treatment of patients with acquired inhibitors. Corticosteroids, cytotoxic drugs such as cyclophosphamide, azathioprine, 6-mercaptopurine, and vincristine, as well as cyclosporine have all been successful in lowering and suppressing inhibitor titers. A prospective, randomized trial reported by Green et al showed that inhibitor titers resolved within 3 weeks in 10 of 30 patients treated initially with prednisone. When randomized to receive continuation of prednisone vs. oral cyclophosphamide vs. a combination of prednisone and cyclophosphamide, half of the remaining 20 patients had resolution of their inhibitor titers regardless of the treatment arm. A lower initial inhibitor titer was predictive of a favorable response to treatment. Shaffer and Phillips reported the successful eradication of inhibitors in 9 consecutive acquired hemophilia patients with a combination of oral cyclophosphamide and prednisone. Subsequent follow-up by both groups of investigators has suggested that patients with lower antibody titers may be sufficiently treated with steroid therapy alone, and the addition of cyclophosphamide may be reserved for those with high titer inhibitors or who fail to respond to steroids. More recently, the use of rituximab has been shown to be effective in some patients with acquired hemophilia. Immunosuppressive therapy is usually continued until the inhibitor disappears and is then slowly withdrawn while patients are closely monitored for return of the antibody.

**Inhibitors in Hemophilia B**

Inhibitors are much less common in hemophilia B, affecting less than 5 percent of patients, but more than 80 percent of these are “high responders”. The development of fIX inhibitors is equally common in patients treated with plasma derived and recombinant fIX products. Several clinical features distinguish fIX inhibitors. More than half of those who develop fIX inhibitors have a history of severe allergic reaction to fIX containing products. In addition, the success rate
of ITI is much lower than for fVIII inhibitors, occurring in only approximately 15 percent of those treated. Furthermore, the development of nephrotic syndrome is seen in a substantial minority of hemophilia B patients undergoing ITI. This is not a recognized complication of ITI with fVIII. Recombinant fVIIa may be the only feasible treatment option for hemophilia B patients who develop inhibitors or who have a history of severe allergic reaction to fIX containing products.

**FUTURE DIRECTIONS**

Gene therapy for hemophilia is clearly on the horizon. This technology will likely reduce or perhaps even eliminate the need for factor replacement therapy for the majority of those affected by hemophilia. However, much work remains in order to bring gene therapy to the forefront of routine hemophilia treatment, and factor replacement therapy is thus expected to remain the mainstay of our clinical management for the foreseeable future. Moreover, gene therapy is not expected to completely eliminate the problem of inhibitors, and these patients will continue to challenge all those who care for them.

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Even though the first successful allogeneic hematopoietic stem cell transplantation occurred in 1959, the clinical practice of bone marrow transplantation was not widely used in this country until the early 1970s. Recently, concepts for the transplantation of hematopoietic stem cells have changed dramatically. High-dose myeloablative conditioning regimens are in the process of being replaced by immunosuppressive regimens which destroy host immune system and neoplastic cells by the co-transplanted donor lymphocytes. Furthermore, the presence of stem cells in the bone marrow, capable of differentiating into a variety of nonhematopoietic tissues as well as the presence of cells in other organs, capable of differentiating into hematopoietic cells, has led to the novel concept of the plasticity of stem cells derived from different tissues. It is anticipated that these remarkable studies will also lead to novel therapeutic strategies.

During the past 30 years, the number of transplant performed has grown exponentially. According to the International Bone Marrow Transplant Registry (IBMTR), approximately 500 institutions worldwide are known to have active transplantation programs, performing more than 17,700 transplantations in 2002. There have been significant changing trends in the practice of stem cell transplantation in the past 5 years. These are: (i) an introduction of nonmyeloablative stem cell transplants makes it safer for older patients to undergo allogeneic stem cell transplantation, (ii) allogenic stem cell transplantation is currently being explored for solid tumors such as renal cell carcinoma and breast cancer, (iii) there has also been a shift in using peripheral blood stem cells and umbilical cord blood as stem cell sources in addition to bone marrow, and (iv) acute myelocytic leukemia has now replaced chronic myelocytic leukemia as the most common indication for allogeneic stem cell transplant, since successful treatment for chronic myelocytic leukemia with Gleevec has greatly decreased the numbers of patients receiving transplants for this disease. Thirteen percent of allograft recipients and 53 percent of autograft recipients are older than 50 years. Two percent of allograft recipients and 20 percent of autograft recipients are older than 60 years. It is the first time in the past decade that the autologous transplant rate has declined. The recently published negative randomized trials in breast cancer have also reduced the autologous stem cell transplants for this disease from 7,000 per year in 1998 to 250 in 2002. Peripheral blood stem cells now account for 90 percent of stem cell source used for autologous transplants in the past few years. With improved supportive care and decreasing costs, the indications for transplantation continue to increase. Selected patients can now receive high-dose therapy with hematopoietic stem cell transplantation as outpatients.

Clinical trials during the past 30 years have demonstrated that high-dose chemotherapy with or without the addition of radiation therapy can result in improved response and overall survival rates for
patients with various malignant and nonmalignant diseases. High-dose chemotherapy enables the clinician to exploit the steep dose-response curves observed with many chemotherapeutic agents. The line representing log kill of malignant cells remains linear or slightly curvilinear for many chemotherapeutic agents, particularly for the alkylating agents. Most of the alkylating agents can be dose escalated 4- to 10-fold; some alkylators, such as thiotepa, can be escalated 30-fold when supported with hematopoietic stem cell transplantation. Most nonalkylating agents cannot be dose escalated more than 2-fold; some exceptions include cytarabine (cytosine arabinoside, ara-C), etoposide, mitoxantrone, and paclitaxel (Taxol). Improved supportive modalities, including antibiotics, antiemetics, and hematopoietic growth factors, and the availability of a variety of blood products have improved the safety of high-dose therapy. However, hematopoietic stem cells derived from bone marrow, peripheral blood, or cord blood or by newer *ex vivo* expansion technologies are required to rescue the patient from myeloablative therapy. Thus, the clinician can continue to escalate the doses of chemotherapy or radiation therapy beyond marrow toxicity to the next level of toxicity, the nonhematologic dose-limiting toxicity.

Although dose escalation is possible with hematopoietic stem cell rescue, not all malignancies can be cured with this treatment modality. In some diseases, the doses necessary to achieve complete tumor cell kill exceed the non-marrow lethal doses of chemotherapy or radiation therapy. In other malignancies, dose escalation beyond the marrow lethal dose results in only modest increases in cell kill. Metastatic melanoma, non-small cell lung cancer, and colon cancer are examples of malignancies that high-dose therapy with hematopoietic stem cell rescue cannot cure.

Even with dose intensification, many patients ultimately suffer disease relapse, which probably results from either failure to eradicate residual tumor cells or, in the case of autotransplantation, the reinfusion of hematopoietic stem cells containing contaminating tumor cells. Using molecular techniques, the latter has been proved to be the case in some hematologic diseases, such as acute myelogenous leukemia (AML) and low-grade lymphoma. In autologous transplant, which is the predominant modality, newer strategies are being developed to improve outcomes using post-transplantation immunotherapeutic approaches to eradicate minimal residual disease and to decrease potential tumor cell contamination by either positive (stem cell selection) or negative (purging) techniques. These approaches are discussed later.

Allogeneic transplantation provides a source of hematopoietic stem cells that is devoid of contaminating tumor cells. Growing clinical experience supports an immunotherapeutic (graft-versus-tumor) effect of the donor immune system to eradicate minimal residual disease after transplant. This immunoreactivity can be exploited following nonmyeloablative stem cell transplant for residual disease or at the time of disease relapse by donor lymphocyte infusions, inducing complete remission in many hematologic malignancies. Unfortunately, transplant-related morbidity and mortality remain problematic because of graft-versus-host disease (GVHD) and prolonged immunosuppression.

**SCIENTIFIC BACKGROUND**

**High-dose Therapy Rationale**

The cytocidal effect of chemotherapy in cell culture and animal models follows first-order kinetics. Each treatment kills a set fraction of cancer cells, irrespective of the starting number. The degree of kill in these experimental systems is dose dependent: tumor cell viability decreases in a logarithmic manner, with a linear increase in drug dose. A modest escalation in the dose may result in a much higher fractional kill of tumor cells. Sublethal chemotherapy selects for and encourages development of resistant cells. The use of several chemotherapeutic agents in combination with different mechanisms of action inhibits the development of resistance. In addition, combinations of agents selected for non-overlapping extramedullary dose-limiting toxicities should be used in maximal doses. Thus, the optimal approach uses the highest possible doses of non–cross-resistant agents with steep dose-response curves as early as possible in the patient’s disease course to achieve the highest tumor cell kill and reduce the development of drug resistance. Eradication of tumor (cure) usually requires an 8- to 12-log kill of cancer cells. A complete clinical remission can be obtained with as little as a 4-log cell kill and a partial remission (50% tumor cytoreduction) with as
little as a 1- or 2-log kill. Complete remissions are the surrogate short-term markers of potentially successful therapy.

The dosages of many active agents are limited by myelosuppression, even with the use of hematopoietic growth factors. The use of hematopoietic stem cell support allows for increased dosage and combination therapy with agents that would normally produce an unacceptable degree of myelosuppression.

**Graft-versus-Tumor Effect**

The eradication of leukemia after allogeneic hematopoietic stem cell transplant (HSCT) results both from the cytotoxic chemoradiotherapy administered prior to transplant and immunologic mechanisms. Two important clinical developments have evolved from identification of this immune-mediated graft-versus-leukemia (GVL) effect. The first is the use of donor lymphocyte infusion (DLI) to treat patients with post-transplant leukemic relapse. With DLI, the majority of patients with recurrent CML after HSCT achieve a complete remission and a smaller but significant fraction of patients with other malignancies respond to therapy. The second is the development of allogeneic HSCT using less toxic nonmyeloablative conditioning regimens. With this approach, low doses of irradiation and chemotherapy, which alone are not sufficient to eradicate tumors, are administered to facilitate graft acceptance and tumor regression is induced by donor immune cells. Nonmyeloablative transplants have significant activity for patients with CML, CLL, myeloma, lymphoma, and renal cell carcinoma. The potent antitumor effects observed after DLI and allogeneic HSCT in a variety of advanced malignancies represent a remarkable demonstration of the curative potential of immunotherapy in contrast to the difficulty of eliciting effective autologous antitumor immune responses to tumor-associated antigens by vaccination or cellular therapy. However, with current approaches to allogeneic HSCT, it has not been possible to separate the beneficial GVL effect from deleterious GVHD. The association of GVL activity with GVHD has implicated donor T-cells reacting with minor histocompatibility antigens expressed by recipient cells as major contributors to the GVL effect. The first clinical demonstration of GVL activity was observed after allogeneic HSCT for advanced leukemia in which the probability of leukemic relapse was found to be significantly lower in those patients who developed acute and/or chronic GVHD. Analysis of patients with leukemia treated with either allogeneic unmodified HSCT, allogeneic T-cell-depleted HSCT, or syngeneic HSCT showed that the risk of relapse was lowest for patients who received allogeneic unmodified HSCT and developed acute and/or chronic GVHD. Transplantation with syngeneic or T-cell-depleted allogeneic marrow to avoid GVHD was associated with a higher risk of relapse unless the conditioning regimen was intensified. However, GVHD is not a prerequisite for GVL activity. A reduction in relapse was evident in the subset of CML and AML patients who received allogeneic unmodified HCT but did not develop GVHD, and remissions have been observed after DLI in the absence of significant GVHD. This suggested that there may be antigenic determinants recognized by T-cells that would permit the separation of GVL responses from GVHD. Other effector mechanisms such as NK cells may also contribute to GVL activity either directly or as a consequence of inflammation induced by allogeneic T-cells.

**Indications for High-dose Therapy with Hematopoietic Stem Cell Transplantation (SCT)**

The current indications for SCT has extended to include elderly patients undergoing allogeneic nonmyeloablative stem cell transplants (NST), and tumor eradication has improved by better conditioning regimens such as radioimmunoconjugates and methods to induce the graft-versus-leukemia effect, such as donor leukocyte infusions (DLI) or allogeneic NST applied after autologous transplants.

**Diseases Treated**

During the past 15 years, the indications for high-dose therapy and hematopoietic stem cell transplantation have changed markedly. The most common indications for allogeneic and autologous transplants differ (Figs 21.1 and 21.2). For acute and chronic leukemias, myelodysplasia (MDS) and nonmalignant diseases (aplastic anemia, immune deficiencies, inherited metabolic disorders), allogeneic transplantation is the predominant approach. Autotransplants are generally used for Hodgkin’s disease, non-Hodgkin’s lymphomas and multiple myeloma and in
small numbers, ovarian cancer and breast cancer. In early 1980, autologous transplants were performed almost exclusively for non-Hodgkin’s lymphoma (NHL) and Hodgkin’s disease. In 1990-2000, breast cancer has been the most common indication for autologous transplant. The recent negative results of several randomized trials in breast cancer have significantly decreased the numbers of autologous transplants performed for breast cancers since late 2000. In 2002, multiple myeloma was the most common indication for autologous transplant, followed by non-Hodgkin’s lymphoma and acute myelogenous leukemia. In addition, a small number of transplants are still being performed for other solid tumors, including breast cancer, ovarian cancer, germ cell cancer and neuroblastoma. The number of allogeneic transplants for nonmalignant diseases has also increased, these include primary immunodeficiencies (severe combined immunodeficiency and Wiskott-Aldrich syndrome), thalassemia major, sickle cell anemia and autoimmune disorders. In the hematologic malignancies, transplantations for multiple myeloma and myelodysplastic syndromes are showing the most rapid rise compared to a decade ago. Although most allogeneic transplantations continue to be performed for acute and chronic leukemias, there has been a recent increase in allogeneic transplantations for immunodeficiency disorders, inherited disorders of metabolism, and inherited erythrocyte abnormalities.

Stem Cell Sources

Bone marrow has been used as the only source of stem cells until the mid-1980s. The introduction of hematopoietic growth factors has made it possible to mobilize the stem cells from the bone marrow into the peripheral blood, resulting in the ability to collect large numbers of stem cells via apheresis and accelerate the engraftment. Currently, peripheral blood stem cells (PBSC) are the major stem cell source accounting for 95 percent of all autologous transplants in adults. Traditionally, allogeneic transplantation used bone marrow grafts. From 1999 to 2002, there was a steady increase in the use of peripheral blood stem cell grafts; this is now the predominant type of graft used in adults. Among children, use of umbilical cord grafts also increased significantly during this time period, though such grafts still account for <20 percent of allotransplants. In addition to differences in their stem cell content, transplants from the three sources differ in the composition and state of activation of immune cells. As a consequence, BM, UCB, and PBSC transplants have different kinetics of hematological recovery, the most rapid engraftment being observed with PBSC and the slowest with UCB. Stem cell source also incurs different risks for developing GVHD: PBSC transplants show similar incidence of acute and a possible increase in chronic GVHD compared with BM. UCB transplants have a favorably low-risk of GVHD even in mismatched transplants.
Stem cell dose is an independent factor in transplants from any source, determining engraftment, transplant-related mortality and risk of leukemic relapse. An understanding of the impact of stem cell source and dose is essential to obtain optimum conditions for a successful outcome after transplant.

**PATIENT ELIGIBILITY**

**Host Factors**

Autologous transplants can be conducted safely in patients up to 75 years of age if they have adequate performance status, physiologic organ function, and hematopoietic stem cells. For allogeneic transplantation, the usual upper age limit is 55 years, although some centers perform HLA-identical sibling transplants in select patients up to 60 to 65 years of age. For unrelated or mismatched related donors, the usual age limitation is 55 years and up to 75 years old in nonmyeloablative allogeneic HSCT. For both autologous and allogeneic transplants, patients must meet a minimum physiologic organ function. Common criteria include pulmonary function tests (forced vital capacity, forced expiratory volume, and corrected diffusing capacity) > 50 percent of predicted; cardiac function with a left ventricular ejection fraction > 40 to 45 percent; no active infections; liver function tests less than two to four times normal; performance status more than 60 percent on the Karnofsky scale or < 2 on the Eastern Cooperative Oncology Group (ECOG) scale; and serum creatinine level of less than 2 mg/dl. In select diseases, patients with impaired renal function can also be considered for autotransplantation (e.g. multiple myeloma patients treated with high-dose melphalan).

**Disease Factors**

In general, patients with malignant disease should show at least a partial response to standard-dose chemotherapy before being considered for high-dose therapy with hematopoietic stem cell transplantation. Some exceptions to this generalization are patients with hematologic malignancies that are refractory to primary chemotherapy and who proceed early in their disease course to transplantation. For example, about 15 to 20 percent of patients with NHL refractory to induction therapy can achieve durable remissions with transplantation.

**CHEMOTHERAPEUTIC AGENTS FOR DOSE-INTENSIVE STRATEGIES**

Agents are chosen for dose intensification based on the steepness and linearity of their dose-response curve; the absence of nonhematologic toxicity that prevents dose-escalation (preferably allowing 5- to 10-fold dose escalation over conventional doses); and, when combined with other agents, a synergistic antitumor effect with a minimum of overlapping nonhematologic toxicity. See Appendix (at the end of the chapter) for description of drugs. The doses of alkylating agents are often reduced to 20 from 40 percent when combined, as compared with use as a single agent in high-dose conditioning regimens. There are a few randomized trials comparing different preparative regimens. Indeed, in a retrospective study of more than 3,500 women with breast cancer undergoing high-dose therapy and autotransplant, more than 20 different preparative regimens were evaluated by multivariate analysis without identification of a statistically superior regimen. Thus, the choice of chemotherapeutic agents is arbitrary, based largely on anecdotal data, and a matter of personal experience and preference. Extramedullary toxicities of the most commonly used conditioning agents are listed in Table 21.1. In most cases, drug doses are limited by gastrointestinal toxicity (mucositis, diarrhea) or major organ toxicity [e.g. heart, lung, kidney, or central nervous system (CNS)]. When combining drugs in a conditioning regimen, particular attention must be given to overlapping toxicities. Pre-existing renal or hepatic insufficiency or both may seriously reduce drug clearance. This can result in higher drug levels and further end-organ toxicity.

**TOTAL-BODY IRRADIATION**

Total-body irradiation (TBI) is an integral component of several conditions/regimens, particularly for hematologic malignancies requiring allogeneic or autologous transplantation. It has been used since the earliest days of bone marrow transplantation for both immunosuppression (prevention of allograft rejection) and antitumor effect. However, the therapeutic ratio of TBI is small. The usual dosage of TBI is 10 to 14 Gy given in twice or thrice daily doses over 3 to 4 days (e.g. 2 Gy b.i.d. for 3 days). Fractionation (and hyperfractionation) substantially reduces the risk of...
Hematopoietic Stem Cell Transplantation for Malignant Diseases

Both interstitial pneumonitis and VOD of the liver. Above that dose, pulmonary, hepatic, and gastrointestinal toxicities become limiting and life-threatening with little therapeutic gain. Acute and chronic toxicities with TBI are summarized in Table 21.2.

Preparative Regimens

During the past 25 years, a large number of intensive preparative (conditioning) regimens requiring hematopoietic stem cell support have been developed. The regimens used for dose intensification are largely empiric, and few have been compared in randomized trials. Important issues, such as optimum combination or doses, the benefit of an "induction" regimen immediately preceding intensification, and the benefit of repeated cycles of dose intensity, have not been rigorously addressed.

Allogeneic Transplant

Preparative regimens must provide effective antitumor activity and suppress host immunity to prevent graft rejection. Commonly used cytotoxic agents include TBI, cyclophosphamide, busulfan, cytarabine, and etoposide. Immunosuppressant agents to reduce the risk of GVHD include steroids, cyclosporine (and cyclosporine analogs, such as tacrolimus), methotrexate, and antithymocyte globulin. Another modality is T-cell depletion of the transplanted cells by monoclonal antibodies, immunoaffinity columns, or immunomagnetic beads. Examples of commonly employed preparative regimens using TBI are shown in Table 21.3.

Reduced Intensity (Non-myeloablative) Allogeneic Transplant

Conventional preparative regimen for allogeneic transplant is characterized by high intensity conditioning, the requirement of prolonged and expensive hospital treatment and a treatment-related mortality (TRM) of 10 to 30 percent depending on diagnosis, disease stage, patient age and donor type. Increasing recognition of the role of graft versus tumor effect has shifted the emphasis from delivery of myeloablative therapy aiming at maximum tumor destruction to optimizing engraftment, thus providing the platform for further adoptive immunotherapy with donor lymphocyte infusion. This has resulted in the emergence of new concepts and procedures that allow replacement of patient bone marrow and immune system with that of the donor by a transplant procedure with markedly reduced intensity of the preparative regimen. This type of transplant is sometimes referred to as mini-BMT, non-myeloablative or reduced intensity transplant. The preparative regimen

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### Table 21.1: Toxicity of common chemotherapeutic agents

<table>
<thead>
<tr>
<th>Drug (dose)</th>
<th>Extramedullary dose-limiting toxicity</th>
<th>Other toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCNU [carmustine] (300–600 mg/m²)</td>
<td>Interstitial pneumonitis</td>
<td>Renal insufficiency, encephalopathy, N/V, VOD</td>
</tr>
<tr>
<td>Busulfan (12–16 mg/kg)</td>
<td>Mucositis, VOD</td>
<td>Seizures, rash, N/V, hyperpigmentation, pneumonitis</td>
</tr>
<tr>
<td>Cyclophosphamide (120–200 mg/kg)</td>
<td>Cardiomyopathy</td>
<td>Hemorrhagic cystitis, SIADH, N/V, interstitial pneumonitis</td>
</tr>
<tr>
<td>Cytarabine [Ara-C] (4–36 g/m²)</td>
<td>CNS ataxia, mucositis</td>
<td>Pulmonary edema, conjunctivitis, rash, fever, hepatitis</td>
</tr>
<tr>
<td>Cisplatin (150–180 mg/m²)</td>
<td>Renal insufficiency, peripheral neuropathy</td>
<td>Renal tubular acidosis, hypomagnesemia, hypokalemia, ototoxicity</td>
</tr>
<tr>
<td>Carboplatin (600–1,500 mg/m²)</td>
<td>Ototoxicity, renal insufficiency</td>
<td>Hepatitis, hypomagnesemia, hypokalemia, peripheral neuropathy</td>
</tr>
<tr>
<td>Etoposide (600–2,400 mg/m²)</td>
<td>Mucositis</td>
<td>N/V, hepatitis, fever, pneumonia</td>
</tr>
<tr>
<td>Ifosfamide (12–16 g/m²)</td>
<td>Encephalopathy, renal insufficiency</td>
<td>Hemorrhagic cystitis</td>
</tr>
<tr>
<td>Melphalan (140–200 mg/m²)</td>
<td>Mucositis</td>
<td>N/V, hepatitis, SIADH, pneumonitis</td>
</tr>
<tr>
<td>Mitoxantrone (30–75 mg/m²)</td>
<td>Cardiomyopathy</td>
<td>Mucositis</td>
</tr>
<tr>
<td>Paclitaxel [Taxol] (500–775 mg/m²)</td>
<td>CNS ataxia, peripheral neuropathy</td>
<td>Anaphylaxis, mucositis</td>
</tr>
<tr>
<td>Thiotepa (500–800 mg/m²)</td>
<td>Mucositis</td>
<td>Intertriginous rash, N/V, hyperpigmentation</td>
</tr>
</tbody>
</table>

CNS: central nervous system; N/V: nausea/vomiting; SIADH: syndrome of inappropriate antidiuretic hormone; VOD: veno-occlusive disease.
Table 21.2: Total-body irradiation-associated acute and chronic toxicities

<table>
<thead>
<tr>
<th>System</th>
<th>Acute symptoms and signs</th>
<th>Acute onset</th>
<th>Chronic symptoms and signs</th>
<th>Onset and incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td>Nausea and vomiting,</td>
<td>24–48 h</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>diarrhea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic Mucosal tissues</td>
<td>Veno-occlusive disease</td>
<td>6–21 d</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Parotitis, decreased</td>
<td>24–48 h</td>
<td>Sicca syndrome, cataracts</td>
<td>20% with fractionation at 0.5</td>
</tr>
<tr>
<td></td>
<td>lacrimation, sore throat,</td>
<td></td>
<td></td>
<td>to 3–4 yr</td>
</tr>
<tr>
<td></td>
<td>mucusitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td>Acute pancreatitis,</td>
<td>7–21 d</td>
<td>Gonadal failure,</td>
<td>&gt;90%</td>
</tr>
<tr>
<td></td>
<td>steroid-induced</td>
<td></td>
<td>hypothyroidism,</td>
<td>40%–55%</td>
</tr>
<tr>
<td></td>
<td>hyperglycemia</td>
<td></td>
<td>delayed bone growth</td>
<td></td>
</tr>
<tr>
<td>Pulmonary Renal</td>
<td>Pneumonitis</td>
<td>1–3 mo</td>
<td>Pulmonary fibrosis</td>
<td>Uncommon</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone marrow transplantation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nephropathy</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Erythema, alopecia</td>
<td>5–10 d</td>
<td>Secondary leukemia,</td>
<td>5%–10%</td>
</tr>
<tr>
<td>Second malignancies</td>
<td></td>
<td></td>
<td>solid tumors</td>
<td>2% at 10 yr; 7% at 15 yr</td>
</tr>
</tbody>
</table>

Table 21.3: Common preparative regimens for high-dose therapy with total-body irradiation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total dose</th>
<th>Daily dose</th>
<th>Schedule (day)</th>
<th>Indications</th>
<th>Autologous or allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single cytotoxic drug regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoxan</td>
<td>120 mg/kg</td>
<td>60 mg/kg</td>
<td>–5, –4</td>
<td>Leukemia, lymphoma,</td>
<td>Both</td>
</tr>
<tr>
<td>TBI</td>
<td>1,200 cGy</td>
<td>200 cGy b.i.d.</td>
<td>–3, –2, –1</td>
<td>aplastic anemia</td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>60 mg/kg</td>
<td>60 mg/kg</td>
<td>–3</td>
<td>Leukemia, lymphoma</td>
<td>Allogeneic</td>
</tr>
<tr>
<td>TBI</td>
<td>1,320 cGy</td>
<td>120 cGy t.i.d.</td>
<td>–7, –6, –5, –4</td>
<td>Leukemia, lymphoma</td>
<td></td>
</tr>
<tr>
<td>Cytarabine (Ara-C)</td>
<td>36 g/m²</td>
<td>3 g/m² b.i.d.</td>
<td>–9, –8, –7, –6,</td>
<td>Leukemia, lymphoma</td>
<td>Allogeneic</td>
</tr>
<tr>
<td>TBI</td>
<td>1,200 cGy</td>
<td>200 cGy b.i.d.</td>
<td>–5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>140 mg/m²</td>
<td>140 mg/m²</td>
<td>–4</td>
<td>Leukemia, multiple</td>
<td>Both</td>
</tr>
<tr>
<td>TBI</td>
<td>1,200 cGy</td>
<td>200 cGy b.i.d.</td>
<td>–3, –2, –1</td>
<td>myeloma</td>
<td></td>
</tr>
<tr>
<td>Combination cytotoxic drug regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine (Ara-C)</td>
<td>18 g/m²</td>
<td>3 g/m² b.i.d.</td>
<td>–8, –7, –6</td>
<td>Leukemia, lymphoma</td>
<td>Allogeneic</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>90 mg/kg</td>
<td>45 mg/kg</td>
<td>–5, –4</td>
<td></td>
<td>Allogeneic</td>
</tr>
<tr>
<td>TBI</td>
<td>1,200 cGy</td>
<td>200 cGy b.i.d.</td>
<td>–3, –2, –1</td>
<td>Leukemia, lymphoma</td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>60 mg/kg</td>
<td>60 mg/kg</td>
<td>–4</td>
<td>Leukemia, lymphoma</td>
<td>Both</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>120 mg/kg</td>
<td>60 mg/kg</td>
<td>–3, –2</td>
<td></td>
<td>Allogeneic</td>
</tr>
<tr>
<td>TBI</td>
<td>1,320 cGy</td>
<td>120 cGy t.i.d.</td>
<td>–8, –7, –6, –5</td>
<td>Leukemia, lymphoma</td>
<td></td>
</tr>
<tr>
<td>Thiotepa</td>
<td>10 mg/kg</td>
<td>5 mg/kg</td>
<td>–5, –4</td>
<td></td>
<td>Allogeneic</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>120 mg/kg</td>
<td>60 mg/kg</td>
<td>–3, –2</td>
<td></td>
<td>Allogeneic</td>
</tr>
<tr>
<td>ATG</td>
<td>120 mg/kg</td>
<td>30 mg/kg</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>1,375 cGy</td>
<td>125 cGy t.i.d.</td>
<td>–9, –8, –7, –6</td>
<td>Leukemia, lymphoma</td>
<td>Allogeneic</td>
</tr>
</tbody>
</table>

a Day 0 is day of transplantation, day –5 is 5 days before transplantation, etc.
b TBI given twice on this day.
TBI, total-body irradiation; ATG, antithymocyte globulin.

used in this type of transplant consists of low-dose TBI of 200 cGy alone or in combination with fludara-
myeloablative transplant, it is frequently necessary to give the recipient post-treatment infusions of additional donor cells. This is referred to as donor lymphocyte infusion, or DLI. However, DLI also carries a significant risk of GVHD development. Immunosuppressive agents used in this type of transplant are combination of oral cyclosporine or tacrolimus with mycophenolate mofetil (MMF), avoiding the debilitating oral mucositis from the use of methotrexate as in conventional SCT. This approach would reduce the toxicity of the transplant procedure and made it possible to treat debilitated patients and possibly extend the use of transplantation to older patients (55–70 years old) or those who are not presently eligible for SCT procedures. Other possible indications include treatment of non-malignant disorders and induction of tolerance for solid organ transplantation. The procedure can be performed in a predominantly outpatient setting. Although a potentially lower level of inflammatory cytokines may be present after nonmyeloablative therapies, fatal GVHD may still occur.

**Autologous Transplant**

In autologous transplant, non-cross-resistant cytotoxic agents with nonoverlapping extramedullary toxicities are often combined. Combination regimens of two or more agents are generally more effective than single-agent regimens; many of the newer regimens rely on the synergistic effect of alkylating agents with agents such as topoisomerase inhibitors. Immunosuppression is not required. Although TBI is used by some centers as part of the preparative regimen for hematologic malignancies (e.g. lymphoma, multiple myeloma), it is avoided in the treatment of solid tumors (e.g. breast, ovary, testicular) because effective tumoricidal doses exceed extramedullary dose-limiting toxicity. Examples of commonly employed preparative regimens using combination chemotherapy are shown in Table 21.4.

**HEMATOPOIETIC STEM CELLS**

Hematopoietic progenitor cells (HPCs) are primitive pluripotent stem cells capable of self-renewal and maturation into any of the hematopoietic lineages and the committed and lineage-restricted progenitor cells. The first observations that lethally irradiated mice could survive after injection of spleen or marrow cells occurred more than 40 years ago. In humans, the first attempts at HPC transplantation began in the late 1960 and early 1970s in recipients of HLA-identical sibling marrow allografts. These initial allogeneic transplants were compromised by severe GVHD. During the subsequent decades, efforts in allogeneic transplantation have been directed toward reducing transplant-related toxicity, decreasing the risk and severity of GVHD, treating relapse with donor lymphocytes and recently the use of “non-myeloablative transplant”. Because technology now permits molecular tagging and tracking of both normal and malignant cells in the blood and marrow, evolving issues in autologous transplant relate to the use of marrow or peripheral blood as a source of HPC and contamination of HPCs by malignant cells. The HPCs have now been characterized in humans to the extent that they can be isolated and expanded in vitro.

Hematopoietic recovery after transplantation (termed engraftment) is believed to occur in two waves: committed progenitor cells repopulating the marrow within the first month, and the true pluripotent stem cells responsible for the delayed but durable component of hematologic recovery. Quantification of the number of HPCs necessary to provide hematopoietic reconstitution has evolved during the past 25 years. Flow cytometry has become the gold standard since the surface marker CD34+ was identified in the late 1980s as being present on HPCs. Patients receiving more than $5 \times 10^6$ CD34+ cells/kg recipient weight have prompt, predictable, and sustained engraftment. There is a growing consensus that more than $2.5 \times 10^6$ CD34+ cells/kg recipient weight is the minimum number of HPCs associated with granulocyte and platelet recovery (ANC > 500, platelets > 20,000) within 14 days after transplantation. The most recent advances in stem cell technology use ex vivo expansion techniques, primarily through cytokine supplementation, to increase the number of hematopoietic stem cells for transplantation. While promising results have been obtained with bone marrow and cord blood, it is becoming clear that novel methods must be developed before cellular therapies using these stem cells can become routine.

Recently, human pluripotent cell lines have been developed from the inner cell mass of human embryos at the blastocyst stage and fetal tissue obtained from
<table>
<thead>
<tr>
<th>Drug</th>
<th>Total dose</th>
<th>Daily dose</th>
<th>Schedule (day)*</th>
<th>Indications</th>
<th>Autologous or allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Big” BU/CY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Busulfan</td>
<td>16 mg/kg</td>
<td>16 mg/kg q.i.d.</td>
<td>–9, –8, –7, –6</td>
<td>Leukemia, lymphoma</td>
<td>Allogeneic</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>200 mg/kg</td>
<td>50 mg/kg</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Little” BU/CY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Busulfan</td>
<td>16 mg/kg</td>
<td>16 mg/kg q.i.d.</td>
<td>–7, –6, –5, –4</td>
<td>Leukemia, lymphoma, myeloma</td>
<td>Both</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>120 mg/kg</td>
<td>60 mg/kg</td>
<td>–3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPB “STAMP I”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>165 mg/m²</td>
<td>55 mg/m²</td>
<td>–6, –5, –4</td>
<td>Breast cancer</td>
<td>Autologous</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>5,625 mg/m²</td>
<td>1,875 mg/m²</td>
<td>–6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>600 mg/m²</td>
<td>600 mg/m²</td>
<td>–3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTCb “STAMP V”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiotepa</td>
<td>500 mg/m²</td>
<td>125 mg/m²</td>
<td>–7, –6, –5, –4</td>
<td>Breast cancer</td>
<td>Autologous</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>6 mg/m²</td>
<td>1.5 g/m²</td>
<td>–7, –6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboplatin</td>
<td>800 mg/m²</td>
<td>200 mg/m²</td>
<td>–7, –6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiotepa</td>
<td>500 mg/m²</td>
<td>125 mg/m²</td>
<td>–7, –6, –5, –4</td>
<td>Breast cancer</td>
<td>Autologous</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>6 g/m²</td>
<td>1.5 g/m²</td>
<td>7, –6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>300-600 mg/m²</td>
<td>300-600 mg/m²</td>
<td>–6</td>
<td>Hodgkin’s disease</td>
<td>Autologous</td>
</tr>
<tr>
<td>Etoposide</td>
<td>900-2,400 mg/m²</td>
<td>300-800 mg/m²</td>
<td>–6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoxan</td>
<td>6-7.2 g/m²</td>
<td>1.5-1.8 g/m²</td>
<td>–6, –5, –4, –3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>300 mg/m²</td>
<td>300 mg/m²</td>
<td>–6</td>
<td>Hodgkin’s disease, lymphoma</td>
<td>Autologous</td>
</tr>
<tr>
<td>Etoposide</td>
<td>800 mg/m²</td>
<td>200 mg/m²</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine</td>
<td>800-1600 mg/m²</td>
<td>200-400 mg/m²</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>140 mg/m²</td>
<td>140 mg/m²</td>
<td>–1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>16 g/m²</td>
<td>4 mg/m²</td>
<td>–6, –5, –4, –3</td>
<td>Lymphoma, testicular cancer</td>
<td>Autologous</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>1.8 g/m²</td>
<td>600 mg/m²</td>
<td>–6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>1.5 g/m²</td>
<td>500 mg/m² b.i.d.</td>
<td>–6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>300 mg/m²</td>
<td>300 mg/m²</td>
<td>–6</td>
<td>Lymphoma, Hodgkin’s disease</td>
<td>Autologous</td>
</tr>
<tr>
<td>Etoposide</td>
<td>800 mg/m²</td>
<td>200 mg/m²</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine</td>
<td>800 mg/m²</td>
<td>200 mg/m²</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoxan</td>
<td>140 mg/m²</td>
<td>35 mg/km²</td>
<td>–5, –4, –3, –2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>200 mg/m²</td>
<td>100 mg/m²</td>
<td>–3, –2</td>
<td>Multiple myeloma</td>
<td>Autologous</td>
</tr>
<tr>
<td>MCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>75 mg/m²</td>
<td>25 mg/m²</td>
<td>–8, –6, –4</td>
<td>Ovarian cancer</td>
<td>Autologous</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>AUC 28</td>
<td>1/5 total dose</td>
<td>–8, –7, –6, –5, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoxan</td>
<td>120 mg/m²</td>
<td>40 mg/m²</td>
<td>–8, –6, –4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBDA/VP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboplatin</td>
<td>2.25 g/m²</td>
<td>750 mg/m²</td>
<td>–6, –5, –4</td>
<td>Testicular cancer</td>
<td>Autologous</td>
</tr>
<tr>
<td>Etoposide</td>
<td>2.1 g/m²</td>
<td>700 mg/m²</td>
<td>–6, –5, –4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC: area under the curve (Calvert formula).

* = days prior to stem cell transplant
terminated pregnancies. These embryonic stem cells (ES), can differentiate to all cell lineages \textit{in vivo}, and can be induced to differentiate to most cell types \textit{in vitro}. Although embryonic stem cells have been isolated from humans, their use in research as well as therapeutics is encumbered by ethical considerations. Other investigators have also demonstrated a surprising plasticity of the human mesenchymal stem cells (MSC) isolated from marrow aspirates. These adult MSC could be induced to differentiate to lineages of mesenchymal cell tissues, including adipoblasts, chondroblasts, or osteoblasts, endothelial cells. Individual stem cells were identified that, when expanded to colonies, retained their multilineage potential. Thus, because MSCs can be selected and expanded under conditions that should be readily adaptable to production by clinical good manufacturing process and are easily transduced with retroviral vectors, they may be an ideal source of cells for therapy of degenerative or traumatic disorders of mesodermal cells or for therapy of single gene disorders.

\textit{Allogeneic transplant} is used mostly for the treatment of leukemia and other hematologic malignancies. Less than 5 percent of allogeneic transplants are used for nonmalignant diseases such as aplastic anemia, immunodeficiency syndromes, or hemoglobinopathies. Although most allogeneic transplants consist of bone marrow donation from an HLA-identical sibling, there is a growing use of PBSCs, unrelated bone marrow donors, mismatched family donors, and umbilical cord blood. Until recently, donors were identified by serologic phenotype testing for class I and class II major histocompatibility complex molecules HLA-A, -B, and -DR on lymphocytes. Mendelian inheritance predicts a 25 percent likelihood of identifying an HLA-identical sibling donor within a family; another 5 percent of patients have a one-antigen–mismatched family donor. Through the efforts of the National Marrow Donor Program (NMDP), which has HLA typing on more than 5.5 million volunteers, an HLA-compatible unrelated donor can be identified for many patients. Because of HLA polymorphism, most transplant centers now perform high resolution HLA typing that includes HLA-C, -DRB1, -DP and -DQ; occasionally, HLA sequenced-based typing is required to confirm compatibility for both class I and class II HLA antigens. This is particularly important in evaluating potential unrelated donors. Using this technology, an acceptable match can be identified in more than 80 percent of Caucasian patients in the NMDP. In contrast, minorities are still greatly under-represented (30%) in the NMDP in spite of their significant growth in the registry in the past 10 years. Therefore, the likelihood of identifying an acceptable match for these patients is considerably lower. Transplant-related mortality rates range from 20 to 30 percent in HLA-identical sibling transplant recipients and is significantly higher in recipients of mismatched unrelated grafts and haploidentical grafts (40–45%), compared with recipients of matched unrelated marrow grafts (23%). Therefore, patients who lack a closely matched family donor should be offered a phenotypically matched unrelated donor if available. There is no apparent advantage to using a mismatched unrelated versus a haploidentical family donor.

GVHD is the most common cause of treatment-related mortality, with significant acute GVHD (first 100 days after transplantation) occurring in 10 to 40 percent of HLA-identical sibling transplant recipients and in more than 40 to 80 percent of unrelated and mismatched marrow recipients. Chronic GVHD (occurring more than 100 days after transplantation) occurs in about 50 percent of HLA-identical sibling transplant recipients; the incidence is higher with unrelated donors and mismatched donors. GVHD prophylaxis requires immunosuppression of the donor immune system: a number of modalities are available, usually in combination, including antithymocyte globulin, methotrexate, cyclosporine and cyclosporine analogs, such as tacrolimus, corticosteroids, T-cell depletion of allografts, and monoclonal antibodies (i.e. OKT3). Although the incidence of severe acute and chronic GVHD is lower with T-cell depletion techniques (5–20%), there is an increase in graft failure and disease relapse. Recent advances in GVHD prophylaxis has extended the potential donor pool to include partially mismatched donors and haploidentical donors.

Another potential donor source is umbilical cord blood. Transplantation of umbilical cord blood was successfully performed for the first time in 1988 to treat a boy with Fanconi’s anemia. By the early 2002, a total of 2,000 umbilical cord blood transplantations have been reported worldwide including 500 adult
recipients (Netcord data). In allogeneic recipients less than 20 years of age without matched sibling donors, the use of umbilical cord blood (UCB) increased from less than 5 percent in 1995 to 15 percent in 2000. The advantages of cord blood as a source of hematopoietic stem cells for transplantation are due to its superior proliferative capacity and lower risk of GVHD. A 100 ml unit of cord blood contains 1/10th the number of nucleated cells and progenitor cells (CD34+ cells) present in 1000 ml of marrow; but because they proliferate rapidly, the stem cells in a single unit of cord blood can reconstitute the entire hematopoietic system. The immaturity of lymphocytes in cord blood dampens the GVHD reaction. A joint European study showed that recipients of cord blood from HLA-identical siblings had a lower risk of acute or chronic graft-versus-host disease than marrow recipients from HLA-identical siblings. Children with acute leukemia who received HLA-mismatched cord blood from an unrelated donor also had a lower risk of graft-versus-host disease than recipients of HLA-mismatched marrow from an unrelated donor. Most studies in children with malignant or nonmalignant hematologic diseases have shown that long-term survival after transplantation of cord blood is similar or superior to survival after transplantation of marrow when the donor is a sibling. Multicenter trials of cord blood transplants in adults reported 90 percent neutrophil engraftment and low GVHD rate despite HLA mismatches. Transplantation-related mortality were related to the number of nucleated cells in the graft and degree of HLA disparity. Patients who received no more than $1 \times 10^7$ nucleated cells per kilogram had a 75 percent probability of death, whereas recipients of at least $3 \times 10^7$ nucleated cells per kilogram had a 30 percent probability of death. Three or more HLA mismatches result in 50 percent mortality rate in adults. An advantage of cord blood over adult marrow for allogeneic transplantation is that the cells are readily available in cord blood banks, are routinely typed for HLA antigens and ABO blood groups, and are tested for infectious agents. This reduces the time required to search for and identify a suitable donor, which is crucial for patients in desperate need of a transplant. The age and weight of the recipient is not an obstacle, as long as the unit of cord blood contains more than $2 \times 10^7$ nucleated cells per kilogram of the recipient’s weight at the time of collection. A simultaneous search of registries of bone marrow donors and cord blood banks for appropriate matches and adequate cell numbers should be initiated for patients without related family donors. In United States, the National Marrow Donor Program (NMDP) provides a single point for both unrelated donor as well as cord blood unit search. Currently, 17,000 cord blood units are available for search through NMDP. The final choice of the source of stem cells must take into account the degree of HLA identity, the availability of the donor, the urgency of transplantation, and the number of cells in the unit of cord blood.

A recent trend in allogeneic transplantation is to use hematopoietic growth factor primed PBSCs. Sufficient numbers of HPCs can usually be collected in one or two apheresis. For allogeneic PBSC transplant, most centers prefer the minimum cell dose to be $5 \times 10^6$. The use of PBSC negates the need for a bone marrow harvest and provides 3- to 4-fold higher number of CD34+ cells and an approximately 10-fold higher total number of lymphoid subsets when mobilized with G-CSF than that obtained from bone marrow. This allows for more rapid engraftment. Even though PBSCs contain a log or more T cells than does bone marrow, the incidence of acute GVHD does not appear to be increased. The incidence of chronic GVHD remains unsettled, it was found to be higher after PBSCT in the prospective and retrospective registry analyses but to be similar in two large prospective studies. Different cytokine and GVHD prophylaxis regimens may contribute to this discrepancy. In two major studies (prospective randomized and retrospective registry data), the disease-free survival rates were higher after PBSCT, especially in patients with advanced-stage disease. Data on immune reconstitution are in favor of PBSCT but discrepant for NK cell reconstitution. The significantly lower incidence of molecular and cytogenetic relapse in patients with CML is indicative of a more pronounced GVL effect after PBSCT. Whether this beneficial GVL effect holds up in more aggressive disease categories remains to be shown. The megadose concept of CD34+ cells, including veto cells, contained in PBSC allografts allows crossing major HLA barriers. It has been proposed that G-CSF-primed BM allografts combine fast cellular reconstitution with an incidence of GVHD similar to steady-state BMT, but the available data are inconclusive.
Autologous transplants and the number of centers performing them are increasing at a striking rate. Currently, most centers use autologous PBSCs as the source of HPCs to support high-dose therapy. With the advent of PBSCs and hematopoietic growth factors, the duration of marrow aplasia has been significantly shortened compared with that of autologous bone marrow transplant. Randomized trials have demonstrated that the use of PBSCs has resulted in fewer infectious complications, shorter hospitalizations, and lower costs. Many centers are performing autologous transplants in the outpatient setting.

PBSCs are collected by leukapheresis. This is usually coordinated with the transplantation center’s blood bank. Patients require insertion of a large-bore central venous catheter before initiation of apheresis. PBSCs can be collected in the steady state or after mobilization by hematopoietic growth factors [e.g. granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF)] with or without chemotherapy (Table 21.5).

Although cyclophosphamide (1.5–7 g/m²) is the most common single chemotherapeutic agent reported in stem cell mobilization regimens, a number of other agents have been used either alone or in combination with cyclophosphamide or with other agents. Several investigators have found that the infusion of at least 2.5 × 10⁶ CD34+ cells/kg resulted in timely hematopoietic recovery. In addition, more recently, it was observed that the infusion of at least 5.0 × 10⁶ CD34+ cells/kg is consistently associated with more predictable and rapid recovery, particularly of platelets. Most patients reach their target CD34+ cell goal within two to five collections. However, in heavily pretreated patients, this minimum requirement is often difficult to achieve. Recent pilot trials indicate that some of the newer hematopoietic growth factors [stem cell factor (SCF), danilepistim, flt-3 ligand], either alone or in combination with other growth factors, increase the yield of CD34+ cells.

PBSC mobilization techniques may also increase the number of tumor cells in the peripheral blood. For example, tumor cells are commonly present in the bone marrow of patients with advanced breast cancer—and may be present even in those with stage I disease. About one-fourth of patients with advanced breast cancer have detectable cells in the peripheral circulation; during stem cell mobilization, significantly higher percentages of patients may have detectable tumor cells. Similar findings but with lower rates of contamination have been reported for lymphoma. Essentially all PBSC collections from patients with multiple myeloma contain contaminating tumor cells. The study of AML with neomycin resistance gene marking study convincingly demonstrates that malignant cells within autograft can survive and grow within a patient after reinfusion.

Efforts to reduce the number of contaminating tumor cells in PBSC autografts have used techniques based on physical, immunologic, and pharmacologic methods. Pharmacologic methods are generally aimed at removing tumor cells from the autograft (purging; negative selection) rather than by enrichment for HPCs. The most common pharmacologic agent is 4-hydroperoxycyclophosphamide. Although promising in pilot trials, the Food and Drug Administration (FDA) has removed this drug from clinical trials. Physical methods (e.g. density gradients and counterflow centrifugal elutriation) are used less commonly: they use cell size, shape, and density to separate cell populations. The most commonly employed separation techniques use immunologic methodology, most often positive selection for CD34+ cells. An anti-CD34+ cell antibody is bound to a solid phase (e.g. immunoaffinity column, immunomagnetic beads), which binds cells, and then the cells are later released. This results in a 2- to 4-log depletion of contaminating tumor cells. One of the most recent advances in positive selection is the use of sequential columns (anti-CD2 followed by anti-CD34) and by combination of immunologic and physical methods (immunoaffinity columns followed by high-speed flow cytometry). The latter method has been reported to result in a 5- to 7-log depletion of tumor cells.

Table 21.5: Relative increase in peripheral blood stem cells using different mobilization regimens

<table>
<thead>
<tr>
<th>Modality</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state</td>
<td>1×</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>10–20×</td>
</tr>
<tr>
<td>Growth factor alone (G-CSF most common)</td>
<td>10×</td>
</tr>
<tr>
<td>Chemotherapy plus growth factor (G-CSF or GM-CSF)</td>
<td>100–1,000×</td>
</tr>
</tbody>
</table>

G-CSF: granulocyte colony-stimulating factor;
A new technology to reduce contaminating tumor cells uses in vitro culture. Small numbers of HPCs can be expanded 3- to 20-fold ex vivo with combinations of cytokines (e.g. IL-3, IL-6, G-CSF, SCF, flt-3, GM-CSF, GM-CSF/IL-3) in large-volume culture or bioreactors. Preliminary results indicate that these expanded cells are capable of complete hematopoietic reconstitution after high-dose therapy. A similar technique using long-term culture permits growth of HPCs while malignant tumor cells are eliminated, allowing for potential autografts in patients with hematologic malignancies, such as CML, who may not be candidates for allogeneic transplantation.

### Hematopoietic Growth Factors and Cytokines

More than 20 different cytokines and growth factors are approved or under investigation for use in hematopoietic stem cell transplantation. Colony-stimulating factors shorten the time to bone marrow or PBSC engraftment after high-dose chemotherapy. They act by binding to specific cell surface receptors stimulating proliferation, differentiation, commitment, and selected end-cell functions. Two commercially available hematopoietic growth factors are G-CSF (filgrastim) and GM-CSF (sargramostim). The most common dosages and indications for these growth factors are listed in Table 21.6. For HPC mobilization with growth factors alone, most clinicians start the growth factor on day 1, with initiation of apheresis on day 5. For chemotherapy plus growth factor mobilization, the growth factor is started on the day after completion of the chemotherapy, and apheresis commences when the white blood cell count is more than 1,000. After transplantation, the growth factors are usually started the same day (day 0) or on day 1; growth factor support is continued daily until the absolute neutrophil count is more than 2,000 for a minimum of 1 day.

Several new hematopoietic growth factors are in clinical trials. These growth factors are designed to improve platelet recovery (IL-11, thrombopoietin, megakaryocyte-derived growth factor), to improve stem cell mobilization in patients who are predicted to be poor mobilizers with G-CSF or GM-CSF (IL-3, daniplestim, stem cell factor), or to enhance dendritic cell proliferation (flt-3 ligand, stem cell factor) as part of immunotherapeutic approaches. Recently, keratino-

cyte growth factor (KGF) has been shown to reduce the incidence of mucositis and graft-versus-host disease in animal models. Preclinical studies have demonstrated that KGF can prevent lung and gastrointestinal toxicity following chemotherapy and radiation, and preliminary clinical data in the later setting supports these findings. In the experimental allogeneic bone marrow transplant scenario, KGF has shown significant ability to prevent graft-versus-host disease by maintaining gastrointestinal tract integrity and acting as a “cytokine shield” to prevent subsequent proinflammatory cytokine generation. Within this setting, KGF has also shown an ability to prevent experimental idiopathic pneumonia syndrome by stimulating production of surfactant protein A, promoting alveolar epithelialization and attenuating immune-mediated injury. Perhaps most unexpectedly, KGF appears to be able to maintain thymic function during allogeneic stem cell transplantation and so promote T cell engraftment and reconstitution. These data suggest that KGF will find a therapeutic role in the prevention of epithelial toxicity following intensive chemotherapy and radiotherapy protocols and in allogeneic stem cell transplantation.

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Clinical indication</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSFa</td>
<td>Peripheral blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPC mobilization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With chemotherapy</td>
<td>5–10 μg/kg S.C.</td>
</tr>
<tr>
<td></td>
<td>Without chemotherapy</td>
<td>10–16 μg/kg S.C.</td>
</tr>
<tr>
<td></td>
<td>Hematopoietic recovery after transplantation</td>
<td>5 μg/kg S.C.</td>
</tr>
<tr>
<td>GM-CSFb</td>
<td>Peripheral blood HPC mobilization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With chemotherapy</td>
<td>250 μg/m² S.C.</td>
</tr>
<tr>
<td></td>
<td>Hematopoietic recovery after transplantation</td>
<td>250 μg/m² S.C.</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Red blood cell recovery after transplantation</td>
<td>150–300 μg/kg S.C. three times per week</td>
</tr>
</tbody>
</table>

a Often rounded off to standard vial size of 300 μg or 480 μg; a common approach is 300 μg for patients who weigh <60 kg, and 480 μg for patients who weigh >60 kg.
Other cytokines under development or in clinical trials in hematopoietic stem cell transplantation are shown in Table 21.7. Many of these have multiple functions.

Toxicities of dose-intensive regimens can be formidable and life-threatening. They vary considerably with the different preparative regimens, type of transplant (autologous versus allogeneic; related versus unrelated versus mismatched), and the patient’s physiologic organ function and performance status. Some of the toxicities associated with transplant preparative regimens are outlined in Tables 21.1 and 21.2. As indicated, some of the toxicities are acute, whereas others are chronic. Stomatitis, esophagitis, and diarrhea can be severe with some regimens. Hepatic, renal, or pulmonary toxicities can occur in 20 to 30 percent of patients. Most patients require blood product support in the peri-transplant period. Central venous catheter infections or thrombosis can be problematic. Most centers use prophylactic antibiotics to prevent bacterial, viral, and fungal infections. One of the most devastating late toxicities is the development of secondary malignancies. The allogeneic transplant recipients were 8.3 times higher risk of new solid cancers than expected among those who survived 10 or more years after transplantation. The cumulative incidence rate was 2.2 percent at 10 years and 6.7 percent at 15 years. In multivariate analyses, higher doses of total-body irradiation were associated with a higher risk of solid cancers. Chronic graft-versus-host disease and male sex were strongly linked with an excess risk of squamous cell cancers of the buccal cavity and skin. Long-term survivors of BMT for childhood leukemia have an increased risk of solid cancers and post-transplant lymphoproliferative disorders (PTLD), related to both transplant therapy and treatment given before BMT. Cumulative risk of solid cancers increased sharply to 11.0 percent at 15 years and was highest among children at ages younger than 5 years at transplantation. Thyroid and brain cancers accounted for most of the strong age trend; many of these patients received cranial irradiation before BMT. Multivariate analyses showed increased solid tumor risks associated with high-dose total-body irradiation and younger age at transplantation, whereas chronic graft-versus-host disease was associated with a decreased risk. Risk factors for PTLD included chronic graft-versus-host disease, unrelated or HLA-desperate related donor, T-cell-depleted graft, and antithymocyte globulin therapy. Hematologic disorders, including myelodysplastic syndrome, lymphoma, and secondary leukemias, have been variously reported in 5 to 15 percent of long-term survivors.

**RESPONSE AND LONG-TERM OUTCOMES**

With a few exceptions, the goal of high-dose therapy with hematopoietic stem cell transplantation is to cure or substantially prolong good-quality survival. The short-term surrogate marker for improved survival or cure is complete remission (CR). Partial remission (PR) rarely translates into important increases in survival and represents only a 1- to 3-log kill of malignant cells. Therefore, partial remission rates have little meaning in dose-intensive regimens. Less than half of patients with advanced malignancy obtain durable remissions with current dose-intensive regimens, stimulating major research efforts to eradicate minimal residual disease after transplantation. The role of dose intensity is covered in disease-related chapters and is presented only in summary form here.

**Acute Myelogenous Leukemia**

Acute myelogenous leukemia (AML) is curable in 15 to 45 percent of patients with standard chemotherapy.
The bone marrow karyotype at diagnosis is not only associated with the response to induction chemotherapy for adult AML but also with outcomes of postremission therapy. AML associated with t(8;21), t(15;17) or inv(16) predicts a relatively favorable outcome. Whereas in patients lacking these favorable changes, the presence of a complex karyotype, -5, del(5q), -7, or abnormalities of 3q define a group with relatively poor prognosis. The remaining group of patients including those with 11q23 abnormalities, +8, +21, +22, del(9q), del(7q) or other miscellaneous structural or numerical defects not encompassed by the favorable or adverse risk groups are found to have an intermediate prognosis. The overall 5-year survival rate based on cytogenetic prognostic groups to be 72, 43 and 17 percent for favorable, intermediate and unfavorable cytogenetic groups respectively. After first relapse, AML is incurable with standard therapy. Cytogenetic analysis is critical for determining which patients are candidates for transplantation as consolidation versus consideration at the time of relapse. Patients with favorable AML subtypes have a more than 80 to 90 percent CR rate and a 50 to 65 percent 5-year survival rate with standard induction and consolidation therapy. These patients are usually considered for transplantation only at the time of disease relapse, although the US intergroup trial suggests that autologous BMT may be useful in favorable AML. In contrast, patients with intermediate cytogenetic risk are often considered for transplantation in the first CR. If there is HLA-matched sibling, allogeneic SCT should be recommended for patients in this group up to age 55 to 65 years. Allogeneic SCT provides the best antileukemic effect due to low relapse rate of 18 percent and 3-year survival rate of 65 percent, although the advantage of allogeneic SCT was not observed in the US intergroup study. Among intermediate risk patients without matched family donor, the 5-year survival after receiving either autologous transplants or high-dose cytarabine is 56 and 48 percent respectively. However, the relapse rate is lower after autologous transplants. It is generally assumed that those patients going on to autologous SCT should receive prior intensive chemotherapy as the best method of in vivo purging. Preliminary reports using PBSC collected after consolidation therapy for autologous transplants results in very low mortality rate and faster engraftment. Several cooperative groups are evaluating the role of gemtuzumab ozogamicin given to high-dose cytarabine or prior to autologous transplants. For patients with unfavorable cytogenetic risk, allogeneic SCT from either family-matched donor or unrelated donor is recommended. The probability of leukemia-free survival is 60 percent in the first CR, 40 percent in the second or later CR, and 20 percent in relapse using HLA-identical sibling donors (IBMTR data) lower rates are observed with matched unrelated donors or mismatched related donors, usually owing to the increased incidence of GVHD. Data from the Fred Hutchinson Cancer Research Center in Seattle indicate that similar results are obtained in patients undergoing transplant during an untreated early relapse with those undergoing transplantation in a second CR. About 10 to 20 percent of patients who fail induction therapy achieve long-term leukemia-free survival with allogeneic transplant.

**Acute Lymphoblastic Leukemia**

Acute lymphoblastic leukemia (ALL) is curable in 60 to 75 percent of affected children but in only 20 to 30 percent of adults. Even in high-risk patients, there are no clinical trials proving that early transplant is beneficial if CR is achieved with standard induction therapy. Because of the rarity of ALL in adults, few institutions have enough patients for randomized trials properly analyzed according to risk factors [e.g. CNS leukemia, high white blood cell count at presentation, male gender, hepatosplenomegaly, Philadelphia chromosome-positive (Ph+) cytogenetics, immunophenotype]. Most clinicians agree, even without substantial clinical trial data, that patients with Ph+ (positive) ALL should proceed to transplant in the first CR. Patients who undergo transplant as consolidation of first CR have a 50 percent leukemia-free survival rate, compared with 40 percent in more than the second CR and 20 percent in relapse using HLA-identical sibling donors; again, lower rates are observed with alternate donors. The outcome of autologous transplantation in ALL is inferior to allogeneic transplants.

**Chronic Myelogenous Leukemia**

Chronic myelogenous leukemia (CML) is no longer the most common indication for allogeneic trans-
Hematopoietic Stem Cell Transplantation for Malignant Diseases

plantation since the clinical trials on imatinib mesylate (STI-571) to treat CML results in major cytogenetic responses in 60 percent of CML patients in chronic phase who had failed interferon. Up until recently, only interferon therapy alone or with cytosine arabinoside was the only non-transplant therapy resulting in major cytogenetic responses in 25 to 40 percent of patients, with a median duration of survival of more than 7 years. Imatinib has demonstrated significant activity in all phases of Philadelphia chromosome (Ph)–positive chronic myeloid leukemia (CML). The recommended dosage is 400 mg daily for chronic phase CML and 600 mg daily for CML in transformation. In patients with previously untreated early chronic phase CML, 400 mg imatinib orally daily has produced complete hematologic responses (CHR) in more than 95 percent of patients, and complete cytogenetic responses in 76 percent compared to 14.5 percent in patients treated with interferon and cytarabine. Among these patients, major molecular response (≥ 3 log reduction in BCR-ABL/BCR level) was seen in 39 percent of all patients treated with imatinib but only 2 percent of all those given interferon plus cytarabine. Achievement of a complete cytogenetic response with interferon-alpha (IFN-α) has been associated with 10-year survival rates of 70 to 85 percent. Patients with undetectable BCR-ABL levels after IFN-α have not relapsed after long-term follow-up. Thus, while achievement of a complete cytogenetic response is an important short-term goal in CML therapy, reaching undetectable levels of BCR-ABL may improve long-term event-free survival.

The ability of allogeneic SCT to produce long-term remission and cure is well established. Cure rates approach 70 percent in patients with CML in chronic phase who receive an HLA-identical sibling transplant within the first year of the disease. Waiting until the development of accelerated phase or blast phase reduces the leukemia-free survival to 35 percent and 15 percent, respectively. In the setting of matched unrelated transplant, about 40 percent of patients are cured when transplantation is performed in chronic phase within the first year. A report from Seattle indicates that the use of interferon for more than 6 months results in an increased incidence of acute GVHD in recipients of matched unrelated donor transplants and, subsequently, in lower survival rates than those seen in patients who did not receive interferon therapy. However, recent reports by the French group as well as IBMTR/NMDP shows no adverse effect of interferon pretreatment on the outcome of subsequent SCT. Patients who relapse after transplant may enter a durable molecular remission with infusion of donor lymphocytes in 80 percent of patients. Escalated dose of DLI starting at CD3+ cells/kg of 1 × 10^7 for matched sibling transplant and 10^6 for matched unrelated donor results in less incidence of GVHD compared to a single large dose of lymphocytes. In an attempt to avoid GVHD from DLI, several centers have initiated DLI trials in which the infused lymphocytes carry a suicide gene, herpes simplex thymidine kinase, which confers sensitivity to ganciclovir. In the event of severe GVHD, administration of ganciclovir should terminate or ameliorate GVHD. There is no evidence that autografts in CML patients prolongs survival. The reduced intensity regimen is being investigated. The decision how best to treat newly diagnosed CML patients and when to offer allogeneic stem cell transplant remains controversial. Most transplant physicians favor offering a trial of imatinib to newly diagnosed CML patients and when to offer allogeneic stem cell transplant to those patients likely to do extremely well with allogeneic transplant and those likely to do poorly with imatinib.

**Chronic Lymphocytic Leukemia**

Chronic lymphocytic leukemia (CLL) is one of the more recent indications for high-dose therapy with hematopoietic stem cell transplantation. The overall survival of CLL patients less than 60 years old who received conventional treatment is 12 years. Patients with advanced disease (Binet C or Rai stages 3 and 4) have median survival of 3 years; only 10 percent can expect to live at 10 years unless they achieve complete remission. Poor cytogenetic finding such as 11q deletion and disease transformation also imply poor prognosis. Both autologous and allogeneic SCT produces 40 to 60 percent overall survival at 4 years. In spite of lower transplant-related mortality of less than 10 percent in autologous SCT, majority of patients receiving autologous SCT relapse. In most instances, the harvested stem cells are contaminated with residual CLL cells. This has prompted the investigation of several in vitro purging methods including an in vivo purging with monoclonal antibody such as...
Compath-1H and Rituximab. With allogeneic SCT, a survival plateau is seen in 57 percent suggesting that these patients may be cured. This confirms a strong graft versus CLL effect in allogeneic stem cell recipients. Moreover, allogeneic SCT can induce sustained complete responses in patients refractory to treatment. Persistent minimal residual disease following allogeneic SCT does not necessarily correlate with leukemia relapse, while it predicts relapse in most autologous recipients. The sensitivity of the disease to treatment, disease status before transplantation, younger age, performance status, use of peripheral blood as source of stem cells, normal cytogenetics and prior therapy with Fludarabine have been associated with better outcome. Due to advanced age of most CLL patients, high transplant-related mortality associated with allotransplantation, and important role of graft versus CLL effect in eradicating disease, several investigators are exploring the use of reduced intensity regimen with DLI. Preliminary results are encouraging with TRM of 15 percent at 2 years. The event-free survival and overall survival at 2 years were 67 and 72 percent respectively with a median follow-up of 2 years. The long-term outcome of these patients need to be established.

**Hodgkin’s Disease**

Hodgkin’s disease is curable with conventional therapy in most patients. Transplantation is an effective modality for primary treatment failure and high-risk patients (e.g. stage IVB), as consolidation, and for patients with disease relapse; long-term disease-free survival is observed in 20 to 30 percent, 60 to 70 percent, and 40 to 50 percent, respectively, for each of these three disease subgroups [Autologous Blood and Marrow Transplant Registry (ABMTR) data]. A variety of preparative regimens have been reported: the BEAM, CBV, and BEAC regimens listed in Table 21.4 are the most commonly employed. In patients receiving nitrosoureas (e.g. BCNU), the clinician must pay particular attention to respiratory symptoms (dry cough, shortness of breath, hypoxia, and interstitial infiltrates on chest radiograph) 4 to 12 weeks after transplant because these symptoms are suggestive of BCNU pulmonary toxicity, a potentially fatal complication that can be reversed with prompt initiation of corticosteroids. A long-term follow-up from Johns Hopkins Oncology Center suggests a lower relapse rate of 34 percent in chemosensitive patients receiving allogeneic transplants versus 51 percent for the auto patients suggesting graft versus Hodgkin’s disease effect. There was a continuing risk of relapse or secondary AML and MDS for 12 years after auto BMT, whereas there were no cases of secondary AML/MDS or relapses beyond 3 years after allo BMT. The allogeneic SCT is usually considered only in the setting of excessive bone marrow involvement or inability to collect sufficient PBSCs for autologous transplant.

**Non-Hodgkin’s Lymphoma**

Non-Hodgkin’s lymphoma (NHL) is curable with conventional therapy in only 30 to 40 percent of patients. Less than 10 percent of relapsed patients achieve long-term survival with conventional salvage therapy. The early transplant trials were conducted in patients with intermediate-grade lymphoma with disease relapse or disease that was refractory to secondary salvage therapy. In this setting, the survival rate was only about 20 percent. Cure rates of 30 to 50 percent were reported in patients who received high-dose therapy with autotransplant earlier in the disease course. A randomized trial comparing high-dose therapy with autologous transplant to standard salvage therapy (DHAP) in patients with chemotherapy-sensitive first relapse proved conclusively that high-dose therapy was the superior treatment (46 percent versus 12 percent 5-year event-free survival rate). Relapse within 12 months from diagnosis, elevated LDH, advanced stage and poor performance status were independent adverse factors for survival and progression-free survival. About 30 percent of patients with primary refractory disease achieve durable remission with high-dose therapy. When transplantation was used as consolidation therapy, a randomized French trial of patients with aggressive NHL did not show significant differences in 3-year or disease-free survivals. However, when the data were retrospectively analyzed focusing on high-risk patients (group 2 or 3 in the International Prognostic Index), HDCT was significantly superior to sequential chemotherapy, with 8-year disease-free survival rates of 55 and 39 percent, respectively. The Italian study also showed superior outcome of HDCT in untreated high-risk IPI patients. Allogeneic transplantation does
Hematopoietic Stem Cell Transplantation for Malignant Diseases

...not appear to be superior to autologous transplantation in treating intermediate-grade lymphomas. Although fewer relapses are observed after allogeneic transplant, presumably because of a graft-versus-lymphoma effect, the transplant-related mortality offsets the lower relapse rate. The use of reduced intensity regimen in NHL has increased one-year overall survival rate after allogeneic HSCT from 23 to 67 percent in one study.

Low-grade NHL accounts for about one third of all lymphomas. These lymphomas are usually extensive at diagnosis and follow an indolent clinical course of 5 to 10 years with or without aggressive therapy. Some centers are treating patients in the first CR with high-dose therapy and autologous transplant. The most favorable results have been observed in patients with minimal disease at the time of transplant whose hematopoietic stem cells are polymerase chain reaction (PCR) negative for bcl-2. This is usually accomplished by \textit{in vitro} bone marrow purging with monoclonal antibody cocktails. However, because of the brevity of the follow-up in most of these reports, the benefit of high-dose therapy with autologous transplant remains uncertain. Most centers now use mobilized PBSC rather than BM in low-grade lymphoma not only to hasten hematologic engraftment, but also because these stem cells are thought to be less contaminated with tumor. Several investigators have begun to explore the use of monoclonal antibodies in stem cell transplantation for patients with lymphoma. These approaches include the development of new high-dose regimens with radiolabeled antibodies, \textit{in vivo} purging techniques with the unlabeled antibodies, and post-transplant adjuvant immunotherapy. More recently, several reports of small numbers of patients from single centers suggest that durable remission can be obtained after allogeneic SCT in low-grade lymphomas. This approach has the advantage of the absence of contaminating tumor cells and a graft-versus-lymphoma effect. Results from the registry data demonstrate that allogeneic BMT is associated with high morbidity and mortality, largely attributable to graft-versus-host disease. In this patient population, however, the probability of relapse appears low with 50 percent disease-free survival rate 3 years after transplant.

One area of controversy is mantle cell lymphomas. These are aggressive intermediate-grade lymphomas with a median survival of about 2 years using conventional therapy. There is currently no definite evidence of a survival advantage using autologous or allogeneic transplant for primary refractory disease, relapsed disease, or after the second CR. Few single institution data reported EFS of 36 to 48 percent at 3 to 4 years. Blastic morphology and heavily pretreated patients are associated with worse prognosis. Few investigators reported encouraging results with the use of rituximab after autologous SCT or using intensive chemotherapy regimen, hyper-CVAD, cytarabine and methotrexate to induce molecular remission followed by allogeneic SCT.

Because most patients with NHL relapse even after high-dose therapy, current emphasis is focused on post-transplant immunotherapy to eradicate minimal residual disease. This includes low-dose IL-2, interferons, idiotype-specific vaccines, and dendritic cell vaccines.

**Multiple Myeloma**

Multiple myeloma is an incurable B-cell malignancy that constitutes 10 percent of all hematologic malignancies. With standard therapy, the median survival is 30 to 36 months. A randomized trial comparing high-dose therapy with autologous transplant to standard chemotherapy demonstrated a superior event-free survival and overall survival in the high dose therapy arm (7-year EFS 16 vs 8% and overall survival time, 57 versus 44 months). More patients in the high-dose arm achieved complete or very good partial response (38 versus 10%), however, there was no plateau of the survival curve. To improve the outcome, subsequent randomized study comparing melphalan 200 mg/M² and melphalan 140 mg/M² plus TBI was initiated. The melphalan 200 mg/M² was significantly less toxic with shorter neutropenia and thrombocytopenia. The melphalan-alone group resulted in better overall survival even though the event-free survival and response rate was the same. Others have shown that tandem stem cell transplants in newly diagnosed patients was safe and increased the complete response rate from 24 percent after the first transplant to 43 percent after two transplants. However, the impact of the tandem...
transplant on the EFS and OS needs further evaluation. In the French randomized trial, double transplant with PBSC appears to be superior to double transplant with marrow and single high-dose therapy in terms of immediate response, EFS and OS. Therefore, the recommended preparative regimen is melphalan 200 mg/M² and the preferred source of stem cell is PBSC. The absence of survival improvement with CD34+ selection in randomized studies in spite of a lower tumor load in the graft confirmed the persistence of the malignant cells in the patients after HDT. Interferon α maintenance appears to prolong EFS and OS for patients responding to HDT in retrospective study, a randomized trial in United States is ongoing. Allogeneic transplant, in contrast, may be curative in 20 to 25 percent of patients but is associated with extremely high transplantation-related mortality rates, approaching 40 to 50 percent in most reports. There is no advantage of allogeneic SCT compared to autologous SCT. However, if transplanted early, about one-third of patients achieving complete remission after allogeneic SCT remain free of disease 6 years later. Several reports have confirmed the durable graft-versus-myeloma effect of donor lymphocyte infusions in relapsed patients postallogeneic SCT. Several ongoing trials are evaluating the role of allogeneic non-myeloablative SCT-alone or following a tumor reduction by autologous SCT. Primary amyloidosis is a plasma cell dyscrasia associated with light-chain deposition in one or more organ systems. With standard therapy, the median survival time is 18 to 24 months, less than 1 year for patients with cardiac amyloidosis. Recent reports from Boston University and the Mayo Clinic indicate that high-dose therapy with autotransplantation can effect high remission rates and improve survival rates. An ECOG trial is now open to evaluate this modality.

Myelofibrosis

Conventional therapies for myelofibrosis are often ineffective with a median survival of 3 to 5 years, it has the worst prognosis of all the chronic myeloproliferative diseases. Recently, a report on 55 patients younger than age 55 years with myelofibrosis who underwent allogeneic HSCT indicated that 48 percent of the recipients of an HLA-identical transplant survived event-free at 5 years. Nevertheless, the 1-year treatment-related mortality in this study was 27 percent in spite of the relatively young age (median, 42 years) of the patients receiving transplants. Further, a recent follow-up from this same group of investigators noted only a 14 percent 5-year overall survival in a subgroup of transplant recipients older than 45 years of age compared to 62 percent for younger patients. Encouraging report of prompt engraftment, significant regression of splenomegaly and marrow fibrosis with reduced intensity regimen in older patients required further investigation in a larger study with myelofibrosis.

Solid Tumors

Over the past two decades, high-dose chemotherapy (HDC) with autologous stem-cell transplantation (ASCT) has been explored for a variety of solid tumors in adults, particularly breast cancer, ovarian cancer
and non-seminomatus germ-cell tumors. The results of prospective phase II studies seemed superior in many cases to the outcome expected with standard-dose chemotherapy (SDC). The value of HDC for adult solid tumors remains, in most instances, a controversial issue, currently under the scrutiny of randomized phase III trial evaluation. ASCT pursuing an immune graft-versus-tumor effect has been evaluated in recent years for patients with advanced and refractory solid malignancies.

Breast Cancer

Breast cancer remains a controversial disease in terms of the value of high-dose therapy (HDC) with ASCT. Several nonrandomized studies demonstrated improved outcomes in patients with primary breast cancer and 10 and more involved axillary lymph nodes after HD-CT. This led to the opinion that high-dose therapy might be the new standard of care for patients with high-risk primary breast cancer. The publication of a small number of randomized studies, that did not show a benefit for the high-dose approach and the case of scientific misconduct, changed the public opinion and practice for HDC and breast cancer in 2000. Recent publication from the German transplant group investigated HDC followed by ASCT compared with standard-dose chemotherapy as adjuvant treatment in patients with primary breast cancer and 10 or more positive axillary lymph nodes. There was a trend in favor of HDC with respect to event-free survival, but without statistical significance. Further follow-up and a meta-analysis of all randomized studies will reveal the effect of HDC as compared with SD-CT as adjuvant treatment in high-risk primary breast cancer.

The MD Anderson group also reported estimated 5-year RFS and OS rates of 62 and 68 percent, respectively after high-dose cyclophosphamide, carmustine, and thiotepa (CBT) regimen plus ASCT as an adjuvant consolidation therapy for high-risk primary breast cancer patients with ≥10 positive axillary lymph nodes after primary surgery or ≥4 positive axillary lymph nodes after neoadjuvant chemotherapy and surgery. For patients with ≥10 positive axillary lymph nodes after primary surgery, the 5-year RFS and OS rates were 71 and 70 percent, respectively, and for patients with ≥4 positive axillary lymph nodes after neoadjuvant chemotherapy, the 5-year RFS and OS rates were 53 and 66 percent, respectively.

Two recently published randomized phase III studies have shown a trend toward improved RFS with HDC in cases of high-risk primary breast cancer. HDC with ASCT in high-risk primary breast cancer at least seems to have the benefit of reducing the risk of disease relapse. This survival benefit was seen, however, only in certain subgroups of high-risk patients, such as patients with a younger age at transplantation. This finding suggests that HDC with AHST is not for every high-risk patient. Identifying the patient populations that will benefit most from this form of treatment has thus become the next major direction in the study of HDC with ASCT as cancer therapy for high-risk primary breast cancer. This benefit will need to be further confirmed in ongoing randomized trials. For now, HDC with AHST in patients with high-risk primary breast cancer has to be confined to clinical trials. Directions for future research on HDC with AHST should include the proper selection of patient groups to receive this kind of treatment and the importance of post-transplantation maintenance therapy, such as hormone therapy in hormone receptor-positive tumors or trastuzumab in HER-2/neu-positive tumors.

Ovarian Cancer

Ovarian cancer, like breast cancer, is sensitive to conventional-dose chemotherapy. Therefore, trials of dose-escalated chemotherapy with hematopoietic stem cell rescue have been pursued. The ABMTR reported the results of 421 patients with ovarian cancer completing high-dose therapy with autologous transplant; the 2-year PFS and OS were 12 and 35 percent, respectively. Favorable prognostic factors included younger age, Karnofsky performance score of at least 90 percent, non-clear-cell disease, remission at transplantation, and platinum sensitivity were associated with better outcomes. Progression-free and overall survivals were 22 and 55 percent, respectively, for women with a high Karnofsky performance score and non-clear-cell, platinum-sensitive tumors. When debulking surgery and platinum-based chemotherapy followed by second look operation was used prior to HDC with melphalan-based regimen, an improvement of the 5-year PFS and OS (29 and 45%) was
observed after a median follow-up of 60 months. Survival is dependent on the residual tumor at second look surgery. Better outcome were obtained in women with a complete pathological response at second look operation with 43 percent 5-year PFS and 75 percent 5-year OS compared to 7 percent survival at 5 years in those with a partial response.

**Germ Cell Cancers**

Germ cell cancers are chemotherapy-sensitive malignancies that afflict young people. Patients with advanced-stage disease who fail to achieve CR to initial platinum-based standard therapy have a poor prognosis: their long-term disease-free survival rate is less than 5 percent. High-dose therapy with autotransplantation in heavily pretreated patients results in a disease-free survival rate of 15 to 20 percent. A second group of patients who are potential candidates for transplantation includes the 10 percent of patients who relapse after achieving CR. Although salvage therapy with vinblastine, ifosfamide and cisplatin (VeIP) produces a more than 50 percent response rate, only 20 to 30 percent of these patients achieve durable remissions. High-dose therapy with autotransplantation results in a 30 to 50 percent long-term disease-free survival rate after autotransplant 5 to 20 percent. A recent updated experience with 65 patients treated with tandem transplant as initial salvage therapy for testicular germ cell neoplasm reported 57 percent of patients are continuously disease-free after the median follow-up of 39 months. Patients in the intermediate and poor prognosis categories tend to do less well. Seventy percent of patients who achieved complete remission after HDCT or after surgery become long-term survivors.

**Renal Cell Cancer**

Metastatic renal cell carcinoma has an extremely poor prognosis, with a median survival of less than one year. Systemic treatment with cytotoxic chemotherapy is usually ineffective. The introduction of interleukin-2 and interferon-alfa for the treatment of metastatic disease provided, for the first time, therapy that induced complete and durable responses. Although some patients who have a complete response to such cytokine-based therapy survive for long periods, the overall rate of response to these agents, either alone or in combination, is usually less than 20 percent. Because renal-cell carcinoma appears to be susceptible to immunomodulation, a graft-versus-tumor effect might be generated after the transplantation of allogeneic lymphocytes from a healthy donor. Non-myeloablative allogeneic transplant using HLA-matched sibling donor has been shown to produce a complete response in 3 of 19 patients and a partial response in 7 of 19 patients. Nine of 19 patients were alive after a median follow-up of 402 days.

**Other diseases** in which high-dose therapy with transplantation has reported efficacy include aplastic anemia (AA) and myelodysplastic syndromes (MDS). AA has a guarded prognosis because of the risk of infection and fatal hemorrhage. The 1-year survival rate for severe AA is less than 20 percent. Allogeneic transplantation results in a long-term survival rate ranging from 50 to 90 percent. Favorable prognostic factors are younger age (<16 years), no prior transfusions, short interval from diagnosis to transplant, and no evidence of infection. The preparative regimen consists of immunosuppressive agents: cyclophosphamide alone or with antithymocyte globulin or with TBI. Patients who do not have a compatible sibling donor may be considered for a matched unrelated donor transplantation. About 15 to 30 percent of patients survive with engraftment.

One of the newest areas of clinical interest is autoimmune diseases. Although predominantly published as case reports, there appears to be clinical improvement or stabilization in disease parameters after high-dose therapy with autologous transplant for multiple sclerosis, systemic lupus erythematosus, scleroderma, and rheumatoid arthritis. Preparative regimens focus on immunosuppression with cyclophosphamide with TBI or antithymocyte globulin.

**FUTURE DIRECTIONS**

The field of high-dose therapy is evolving into ex vivo HPC expansion, gene therapy, improved CD34+ selection techniques, minitransplants, improved GVHD prophylaxis and treatment, improvement in safety of matched unrelated and mismatched donor transplants, DNA and idiotype vaccines, dendritic cell recruitment and transplantation, novel hematopoietic
growth factors, and post-transplant immunotherapy to eradicate minimal residual disease. Preliminary clinical experience suggests that a graft-versus-tumor effect, analogous to the graft-versus-leukemia effect, may be generated against solid tumors such as renal cell cancer, breast cancer, and other malignancies. The use of nonmyeloablative, immuno-suppressive conditioning regimens in solid tumor is under investigation.

SELECTED READINGS


### APPENDIX

Appendix: Agents commonly used in transplant

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Pharmacokinetics</th>
<th>Drug interactions</th>
<th>Toxicity</th>
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<tr>
<td><strong>Alkylators</strong></td>
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<tr>
<td>Carmustine (BCNU)</td>
<td>The medication is hydrolyzed to a chloroethyl diazonium ion, which can chloroethylate DNA and undergo a subsequent reaction to form cross links, and to a substituted isocyanate that can coarabamoylate intracellular molecules</td>
<td>The drug undergoes extensive oxidation by hepatic N-demethylation enzymes to active and inactive products. This is lipid soluble and crosses the blood-brain barrier, they are extensively distributed in the body, including breast milk. Excretion occurs primarily via kidneys. Plasma half-life is approximately 15–20 minutes</td>
<td>Cytotoxicity may be increased by several drugs including cimetidine, amphotericin-B, radiation sensitizers misonidazole, acetylatedronic acid analogues of 3-nitropyrazole, 2-nitroimidazoles and α-difluoromethylornithine</td>
<td>Delayed and cumulative myelosuppression, local pain at the injection site, nausea and pulmonary toxicity are the predominant side effects. At high doses pulmonary and hepatic toxicity are dose limiting. Doses exceeding 1,400 mg/m² are associated with acute or late pneumonitis in at least 20 percent patients. It is also occasionally associated with an increase in incidence of veno-occlusive disease (VOD)</td>
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<tr>
<td>Busulfan</td>
<td>It is a classic bifunctional alkylating agent with phase nonspecific effects on the cell cycle. It causes intrastrand type of DNA cross-linking</td>
<td>Oral absorbed, subsequent blood levels are linearly related to dose. Drug is extensively and rapidly metabolized through conjugation with glutathione to form thiophenium ion. Plasma half-life is about 2.5 hours. The drug rapidly enters CSF</td>
<td>Concomitant busulfan and thioguanine therapy can cause esophageal varices and hepatic toxicity (4%). Phenytoin alters busulfan pharmacokinetics at high dosage and may inhibit its effectiveness</td>
<td>Dose-limiting myelosuppression is the predominant toxicity. Gastrointestinal distress and mucositis can develop. Reversible cholestasis jaundice occasionally develops. About 20 percent of adult BMT patients may develop veno-occlusive disease. The drug may cause fragility and anhidrosis of skin, hyperpigmentation, alopecia and a variety of skin rashes. Hypotension, endocardial fibrosis and intra-alveolar pulmonary fibrosis have been reported. BMT patients may develop dizziness, confusion and seizures. Cataracts and blurred vision can occur. Sterility is common, gynecomastia and adrenal insufficiency may also occur. The drug is carcinogenic, with well-documented cases of leukemia and other tumors</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>Cytoxan acts as a classic alkylating agent. It is considered as cell cycle phase nonspecific, resulting in apoptosis.</td>
<td>It is metabolized by cyto-P-450 enzyme system in the chrome liver to active and inactive metabolites. These metabolites enter cells, where they are degraded to inactive metabolites by aldehyde dehydrogenase to cytotoxic metabolites phosphoramidate mustard and acrolein. The drug is equally effective by oral and intravenous routes of administration. Cyclophosph-</td>
<td>It can inhibit pseudocholinestrase, leading to an increased risk of apnea during anesthesia using succinylcholine. It can inhibit cholinesterase, leading to increased toxicity from cocaine. Risk for cardiotoxicity is increased in patients treated with combination of cyclophosphamide and high-dose cytarabine or anthraclyline. Cimetidine</td>
<td>Myelosuppression and hemorrhagic cystitis are the usual dose limiting toxicity. Nausea and vomiting are common and occasionally diarrhea and hemorrhagic colitis can occur. Alopecia is frequent, pigmented fingernails or skin and dermatitis may occur. Cardiac toxicity including fatal hemorrhagic myocarditis or pericardial effusions may</td>
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<tr>
<th>Drug</th>
<th>Mechanism of action</th>
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<tr>
<td>Ifosfamide</td>
<td>Mechanism of action is similar to cyclophosphamide. It is also considered cell cycle phase, nonspecific</td>
<td>Metabolic transformations are similar to cyclophosphamide, however, the relative rate of these metabolic changes differs. Plasma clearance of the drug is schedule dependent. With large single doses, the terminal half-life is about 16 hours. With divided doses, the half-life is about 7 hours. There is only modest penetration in the CNS. More of the drug (50%) is excreted unchanged in the urine</td>
<td>Acetylcysteine and mesna decrease the urotoxicity of ifosfamide. Cisplatin may increase the neurotoxicity or nephrotoxicity of the drug. Sedatives such as lorazepam and opiates may increase the neurotoxicity of the drug</td>
<td>Myelosuppression is dose related. Nausea, vomiting is of moderate degree, anorexia, diarrhea and constipation occur rarely. Alopecia is common. Rare effects include urticaria, phlebitis and stomatitis. CNS toxicity has been reported in numerous trials with an average incidence of 12 percent. This usually takes the form of a reduced level of arousal, but this can progress through somnolence through coma and even death. Hemorrhagic cystitis is a prominent side effect, high-dose ifosfamide can cause renal failure. Cardiac toxicity can occur at very high doses. Electrolyte imbalance and SIADH may occur. The drug may cause sterility. The drug is considered a potential carcinogen.</td>
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<tr>
<td>Melphalan</td>
<td>The predominant effect is interstrand cross-linking of DNA</td>
<td>Oral absorption is erratic, with bioavailability varying from 32 to 100 percent. The drug undergoes spontaneous degradation to one of 2 hydrolysis products. It is extensively bound to serum albumin. Plasma half-life is 1.5 hours. The drug can cross the blood-brain barrier via the neural amino acid transporter</td>
<td>Severe renal failure has been reported in patients receiving cyclosporine after intravenous melphalan. Intravenous melphalan may reduce the threshold for carmustine-induced pulmonary toxicity. Cisplatin-induced renal dysfunction may alter the pharmacokinetics of melphalan and cause increased toxicity. Nalidixic acid and intravenous melphalan given together may increase the incidence of severe hemorrhagic necrotic enterocolitis in pediatric patients</td>
<td>The dose-limiting toxicity is myelosuppression. High-dose melphalan frequently causes nausea and vomiting. Diarrhea and veno-occlusive disease of the liver are rare. Hypersensitivity reactions are rare. Mucositis, alopecia, skin ulceration are rare. Vasculitis, pulmonary fibrosis, interstitial fibrosis, SIADH and cataracts can also rarely occur. Infertility and menstrual irregularities are common. The drug is mutagenic and a definite leukemogen in humans</td>
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### Hematopoietic Stem Cell Transplantation for Malignant Diseases

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<tr>
<th>Drug</th>
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<tr>
<td>Platinum compounds</td>
<td>The drug covalently binds to DNA bases, leading to predominant intrastrand cross-linking and disruption of DNA function</td>
<td>The drug is widely distributed in the body although it does not enter the CSF. Plasma clearance is biphasic, with the initial half-life of 25–49 minutes and a terminal half-life of 58–73 hours. The drug is metabolized in the liver and the principal route of excretion is the kidney</td>
<td>The cytotoxicity of the drug can be decreased by thio compounds. The toxicity of the drug is increased by drugs that influence renal function such as aminoglycoside antibiotics. Ototoxicity may be increased by the concurrent use of high-dose cytarabine or other ototoxic drugs. Phenytoin levels may be decreased by the medication</td>
<td>Vomiting, renal dysfunction and mild-to-moderate peripheral neuropathy are the predominant forms of toxicity at standard doses. Myelosuppression, peripheral neuropathy and renal dysfunction are the main dose-limiting toxicities. Tinnitus or high frequency hearing loss occurs in about 30 percent of patients. Other rare side effects include cardiotoxicity and decreased peripheral vision. The drug may cause reduced fertility and is carcinogenic.</td>
</tr>
<tr>
<td>Thiotaepa</td>
<td>This acts as a trifunctional alkylating agent and is cell cycle phase nonspecific</td>
<td>It is erratically absorbed from the gastrointestinal tract and from the serosal surfaces. The plasma half-life short (about 1.5 hours)</td>
<td>Increased neuromuscular blockade with prolonged respiratory depression occurred in one patient</td>
<td>Myelosuppression is the dose-limiting toxicity. Mild nausea and vomiting. Local pain can occur at the injection site. Alopecia, hives, rash and pruritus can occur. Headache, dizziness, lower extremity weakness, pain and paresthesia can occur. Decreased fertility can occur.</td>
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<tr>
<td>Nonalkylators</td>
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<tr>
<td>Cytarabine</td>
<td>Cytarabine is actively transported into the cell and activated by an enzyme deoxyctydine kinase to 5'-triphosphate ara-C. This active metabolite can damage DNA by multiple mechanisms</td>
<td>Cytidine deaminase is found in high concentrations in the gastrointestinal mucosa, liver and granulocytes. The terminal half-life is 2–2.5 hours and the drug is excreted in the urine as the inactive metabolite uracil arabinoside. The drug is cleared more slowly from the CSF (2–11 hours) because of paucity of cytidine deaminase in CSF</td>
<td>The elimination of cytosine arabinoside triphosphate, a neurotoxic metabolite, may be decreased by nephrotoxic drugs. The absorption of digoxin may be reduced by the drug. Numerous drugs may enhance antitumor activity. These include thymidine, tetrahydrouridine, uracil arabinoside, IL-3, hydroxyurea, GM-CSF, dipyridamole</td>
<td>Myelosuppression is the dose-limiting toxicity. At high doses, central and peripheral neuropathy, cerebellar ataxia, hemorrhagic conjunctivitis, keratitis, severe gastrointestinal ulceration, severe pulmonary distress and hepatic toxicity occur.</td>
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<tr>
<td>Etoposide</td>
<td>It causes single-stranded breaks in DNA as well as other forms of DNA damage</td>
<td>It is a schedule dependent drug that is available for both oral and intravenous administration. Plasma half-life is variable with values of 3–11 hours. The drug is protein bound and undergoes hepatic metabolism to a variable extent. The major excretory route is renal clearance</td>
<td>Radiosensitization can occur. Low-dose methotrexate, trimetrexate, dipyridamole and cyclosporine increase the cytotoxic effects of etoposide</td>
<td>Major dose-limiting toxicity is myelosuppression but potentially lethal hypersensitivity reactions have been reported in 1–2 percent of patients. Nausea, vomiting, anorexia and diarrhea are common. Mild hepatic dysfunction occurs rarely. Acute transient parotitis has been reported. Alopecia, stomatitis, hyperpigmentation can occur. Rapid administration can cause transient hypotension in 1–2</td>
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<tr>
<td>Mitoxantrone</td>
<td>The drug intercalates into DNA and causes inter- and intrastrand crosslinking</td>
<td>Following intravenous administration, the drug disappears rapidly from the plasma and is widely distributed and bound to the tissues. The drug is excreted as unchanged drug and as 2 major metabolites. Both renal excretion (6–11% over 5 days) and hepatic elimination (25% over 5 days) are limited and overall elimination of the drug is slow (mean half-life 24–37 hours).</td>
<td>It increases the cytotoxicity seen with both radiation therapy and hyperthermia and it may cause synergistic cytotoxicity in combination with cytarabine and tumor necrosis factor</td>
<td>Myelosuppression is the major short-term toxic side effect while cardiac toxicity is the major limiting factor for long-term treatment. Other common effects include nausea, vomiting, mucositis and alopecia. The drug can induce both acute and chronic heart failure. The risk is 2.6 percent with a cumulative dose of ≤140 mg/m², but it rises to 13 percent when the dose exceeds this level. It causes a bluish green discoloration of the sclera, nails and urine. Allergic reactions occur rarely, headache and seizures can occur. It causes reduced fertility</td>
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<tr>
<td>Paclitaxel</td>
<td>It is a novel microtubule inhibitor that works by stabilizing intracellular microtubules in susceptible cells</td>
<td>The drug is metabolized in the liver by cytochrome P-450 isozymes. It is avidly bound by proteins and 1–13 percent is excreted in the urine as unchanged drug. It is widely distributed in the body and has biphasic plasma clearance, with a terminal elimination half-life that varies from 5 to 17 hours.</td>
<td>The drug contains a polyoxyethylated castor oil vehicle. Patients sensitive to this are also sensitive to the medication. The drug is metabolized by cytochrome P-450 enzymes that are inducible by barbiturates and benzodiazepines. Combination with cisplatin results in excessive toxicity. Ketoconazole can inhibit the metabolism of paclitaxel. Combining vinorelbine and paclitaxel results in an increased rate and severity of neurotoxicity</td>
<td>Dose-limiting toxicities include myelosuppression, hypersensitivity reactions, arrhythmias and neuropathy. Patients may develop mild-to-moderate nausea and vomiting, diarrhea, anorexia and change in taste. Rarely paralytic ileus and typhilitis can occur. Minor hepatic function abnormalities can occur. Mild-to-moderate mucositis can occur. EKG changes occur frequently, but severe changes are rare. Ventricular tachycardia and ischemia occur infrequently. Peripheral neuropathy is common. Myalgias and arthralgias are common</td>
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<tr>
<td>Fludarabine phosphate</td>
<td>The drug inhibits DNA synthesis by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase</td>
<td>The drug is distributed widely in the body. The elimination of the drug is either biphasic or triphasic at standard doses. The initial and delayed half-lives were reportedly 36 minutes and more than 9 hours. Renal elimination accounts for about 24 percent of the drug excreted in 24 hours.</td>
<td>Concomitant use with pentostatin can cause severe and even fatal pulmonary toxicity. Fludarabine potentiates the conversion of intracellular cytarabine to its active metabolite, potentially augmenting its effect</td>
<td>Myelosuppression is the most important and dose-limiting toxicity. Other important side effects include fever, chills, infection, nausea, vomiting, malaise, fatigue, anorexia, weakness and in patients with CLL severe hemolytic anemia. At high doses, severe neurotoxicity occurs including</td>
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blindness and death in some patients. Mild-to-moderate neurotoxicity occurs in 21-69 percent of patients. These include weakness, pain, malaise, fatigue, paresthesia, visual disturbances, hearing abnormalities, sleep disorders or headache. Edema and angina have been reported in 19 and 6 percent of patients. Adverse pulmonary effects including pneumonia, cough, dyspnea, allergic pneumonitis or hemoptysis may occur. Rare effects include stomatitis, abnormal liver function tests, cholelithiasis, liver failure and pancreatitis.

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Approximately 70 percent of patients with life-threatening diseases treatable with allogeneic blood stem cell transplantation do not have matched related donors. The National Marrow Donor Program® (NMDP) was established in 1986 to provide human leukocyte antigen (HLA) matched, volunteer unrelated donors for these patients. The NMDP performs this task by maintaining a Registry of more than 5.5 million volunteer donors of marrow and peripheral blood stem cells (PBSC) and 15 cord blood banks containing more than 36,000 units of umbilical cord blood.

The NMDP is a nonprofit organization that operates the congressionally mandated National Bone Marrow Donor Registry under contract from the Health Resources Services Administration, a division of the US Department of Health and Human Services. The NMDP Registry is the largest, most diverse registry of potential stem cell donors in the world. To carry out its mission of providing HLA-matched stem cells for patients, the NMDP operates a worldwide network of medical organizations that cooperate together to locate, procure, and transport stem cells to waiting patients. The wide range of medical organizations in the NMDP network are shown in Table 22.1. The NMDP also provides resources for patients and physicians, collects patient outcome data, and conducts research to improve the outcomes of stem cell transplantation.

A graphical representation of the NMDP network is shown in Fig. 22.1. The National Coordinating Center of the NMDP, located in Minneapolis, Minnesota, houses the computerized NMDP Registry of unrelated stem cell donors, including data on the inventories of NMDP-affiliated cord blood banks. Searches of the NMDP Registry for HLA-matched donors or umbilical cord blood units are accomplished through secure electronic communications between the NMDP and its network of donor centers, apheresis centers, collection centers, transplant centers, and HLA laboratories.

**THE NMDP REGISTRY**

Stem cell transplants, whether related or unrelated, require precise human leukocyte antigen (HLA) matching between donor and patient. Because HLAs are inherited, patients are more likely to find a matching donor within their own racial or ethnic communities. To provide patients of every ethnic community a better chance at finding a matched donor, the NMDP has ongoing recruitment programs to bring more African American, American Indian/Alaska Native, Asian/Pacific Islander, and Hispanic donors to the NMDP Registry. Currently, approxi-
Unrelated Donor Stem Cell Transplantation: The Role of the National Marrow Donor Program

approximately 30 percent of the volunteer donors listed in the NMDP Registry are from racial and ethnic minority groups.

The NMDP Cord Blood Program

The NMDP currently lists more than 36,000 units of donated umbilical cord blood (UCB) from a growing partnership with cord blood banks. The addition of cord blood banks to the NMDP Network was vital to provide more transplant options for patients. All UCB units at NMDP Cord Blood Banks are listed on the Registry and are automatically included in every patient search. The NMDP’s cord blood program is a clinical trial developed in 1998 under an Investigational New Drug (IND) application with the US Food and Drug Administration (FDA). The NMDP continues to work with existing cord blood banks to establish new contacts and list more UCB units on the NMDP Registry.

The NMDP Office of Patient Advocacy

The NMDP’s Office of Patient Advocacy (OPA) works with patients to remove barriers to obtaining an unrelated donor transplant. The OPA connects patients to transplant-related resources, helps patients find a transplant center, or assists them with financial and insurance matters. In addition, the OPA assists patients, their families and physicians with any concerns or questions they may have regarding an NMDP-facilitated search and stem cell transplant.

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**Table 22.1: Organizations in the NMDP network**

<table>
<thead>
<tr>
<th>NMDP participant</th>
<th>Number</th>
<th>Principle function(s)</th>
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<tbody>
<tr>
<td>Donor Centers</td>
<td>87 (7 international)</td>
<td>Recruit volunteers, manage donors</td>
</tr>
<tr>
<td>Cooperative Registries</td>
<td>19 (all international)</td>
<td>International stem cell registries with which the NMDP has reciprocal search privileges</td>
</tr>
<tr>
<td>Recruitment Groups</td>
<td>9</td>
<td>Recruit volunteers to join NMDP Registry</td>
</tr>
<tr>
<td>Apheresis Centers</td>
<td>86 (7 international)</td>
<td>Collect PBSCs from NMDP donors</td>
</tr>
<tr>
<td>Cord Blood Banks</td>
<td>15 (1 international)</td>
<td>Collect and store umbilical cord blood units for transplantation</td>
</tr>
<tr>
<td>Collection Centers</td>
<td>103 (17 international)</td>
<td>Collect marrow from NMDP donors</td>
</tr>
<tr>
<td>Transplant Centers</td>
<td>155 (39 international)</td>
<td>Search Registry, perform stem cell transplants</td>
</tr>
<tr>
<td>DNA Typing Laboratories</td>
<td>23</td>
<td>Perform DNA-based HLA tissue typing of donors</td>
</tr>
<tr>
<td>Research Sample Repository</td>
<td>1</td>
<td>Store paired donor and recipient blood samples for transplant outcome research</td>
</tr>
<tr>
<td>DNA Sample Repositories</td>
<td>3</td>
<td>Store volunteer donor blood samples to be used for high-resolution HLA typing</td>
</tr>
</tbody>
</table>

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**Fig. 22.1: The NMDP Network.** The National Marrow Donor Program (NMDP) Registry can be searched for human leukocyte antigen (HLA)-matched donors or umbilical cord blood units through secure electronic communications between the NMDP Coordinating Center and a network of donor centers, cord blood banks, apheresis centers, collection centers, transplant centers, and HLA laboratories.
The NMDP Research Program

The NMDP collects detailed medical data on every patient who receives a transplant from an NMDP donor. These data are part of a comprehensive research database which the NMDP maintains to assist medical researchers in the field of unrelated stem cell transplantation. The NMDP Research Program develops and promotes research aimed at increasing opportunities for and improving outcomes of unrelated donor stem cell transplants. NMDP resources available to researchers include:

- The largest database of HLA-typed individuals (more than 5.5 million volunteer donors) in the world.
- Outcome, histocompatibility data, donor search and donation side effects data on approximately 90 percent of the more than 20,000 unrelated donor stem cell transplants the NMDP has coordinated since 1987.
- The largest unrelated stem cell donor and recipient HLA database in the world (more than 10,000 paired samples).

In 2004, the NMDP joined with the International Bone Marrow Transplant Registry and Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR) to form the Center for International Blood and Marrow Transplant Research (CIBMTR). A primary focus of the CIBMTR is to coordinate prospective, multicenter trials to increase the safety and success of transplantation. Other activities include coordinating research in immunobiology, providing biostatistics expertise to help researchers interpret and present their transplant-related data, and conducting retrospective studies using NMDP and IBMTR databases and the NMDP’s tissue sample repositories.

The NMDP is one of three partners (along with the CIBMTR and the EMMES Corporation) operating the Blood and Marrow Transplant Clinical Trial Network (BMT CTN). The BMT CTN was established in October 2001 to conduct large multi-institutional clinical trials. The trials will address important issues in hematopoietic stem cell transplantation, thereby furthering understanding of the best possible treatment approaches. Additional goals of the BMT CTN include developing consensus guidelines for diagnosing, monitoring and grading important transplant-related endpoints, and the development and use of novel study designs to increase the efficiency and scientific validity of clinical trials in blood and marrow transplantation.

The NMDP and Unrelated Donor Stem Cell Transplantation

Approximately 75 percent of the stem cell transplants facilitated by the NMDP are for patients with some form of leukemia. Indications for unrelated donor stem cell transplant are constantly changing. Since 1999, NMDP facilitated transplants for adults with acute myelogenous leukemia have increased 66 percent and transplants for myelodysplastic syndromes have increased by 50 percent. In the two years following the FDA approval of imatinib mesylate (Gleevec) to treat chronic myelogenous leukemia (CML), NMDP-facilitated transplants for CML decreased by 45 percent. However, since 2003, the number of NMDP transplants for CML have increased and are now approaching the pre-imatinib mesylate rate.

Current research is exploring the use of reduced-intensity (nonmyeloablative) regimens in allogeneic stem cell transplantation. This has resulted in an increase in the number of stem cell transplants for non-Hodgkin's lymphoma, multiple myeloma, and hemoglobinopathies such as sickle cell disease. Researchers are also investigating the use of stem cell transplantation in malignancies such as breast cancer and renal cell carcinoma. Table 22.2 shows the diseases for which unrelated stem cell donor transplants have been performed by transplant centers in the NMDP network.

Obtaining an NMDP-facilitated Stem Cell Transplant

Initiating a preliminary search of the NMDP Registry to locate an HLA-matched donor or UCB is free and available to any physician. Interpreting the results of a preliminary search and efficiently moving forward through the many steps to a transplant requires an understanding of the clinical aspects of stem cell transplantation and knowledge of the operations of the NMDP.
Lymphocytes differentiate between self and non-self cells by examining the HLA antigens expressed on the surface of cells. To prevent graft rejection and other post-transplant complications, stem cell donors and recipients must be closely HLA matched. The HLA antigens are encoded on the short arm of human chromosome 6, on a segment called the major histocompatibility complex (MHC). There are 3.5 million bases in the MHC, but only a small number are currently matched in stem cell transplantation. The relevant portions of the MHC are further divided up into two regions, class I and class II. Class I antigens (HLA-A, -B, and -C) and class II antigens (HLA-DRB1, -DP, and -DQ) are the antigens most frequently examined when matching potential stem cell donors and transplant recipients.

The NMDP requires that donors and patients have no more than one-antigen mismatch at the HLA-A, -B or -DRB1 locations. Because there are two HLA antigens at each of these three locations, a perfect match is referred to as a 6 of 6 match, and a one-antigen mismatch is termed a 5 of 6 match. Using this terminology, the NMDP will allow a 5/6 match or a 6/6 match for marrow and peripheral blood stem cell transplantation.

**Table 22.2: Diseases treatable by stem cell transplantation**

<table>
<thead>
<tr>
<th>Acute Leukemias</th>
<th>Inherited Immune System Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Lymphoblastic Leukemia (ALL)</td>
<td>Ataxia-Telangiectasia</td>
</tr>
<tr>
<td>Acute Myelogenous Leukemia (AML)</td>
<td>Kostmann Syndrome</td>
</tr>
<tr>
<td>Acute Biphenotypic Leukemia</td>
<td>Leukocyte Adhesion</td>
</tr>
<tr>
<td>Acute Undifferentiated Leukemia</td>
<td>Deficiency</td>
</tr>
<tr>
<td>Chronic Leukemia</td>
<td>DiGeorge Syndrome</td>
</tr>
<tr>
<td>Leukemia (CML)</td>
<td>Bare Lymphocyte Syndrome</td>
</tr>
<tr>
<td>Chronic Myelogenous Leukemia (CML)</td>
<td>Omenn's Syndrome</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukemia (CLL)</td>
<td>Severe Combined</td>
</tr>
<tr>
<td>Juvenile Chronic Leukemia</td>
<td>Immunodeficiency (SCID)</td>
</tr>
<tr>
<td>Myelodysplastic Syndromes</td>
<td>SCID with Adenosine</td>
</tr>
<tr>
<td>Refractory Anemia (RA)</td>
<td>Deaminase</td>
</tr>
<tr>
<td>Refractory Anemia with Excess Blasts</td>
<td>Absence of T &amp; B Cells SCID</td>
</tr>
<tr>
<td>Refractory Anemia with Ringed Sideroblasts (RARS)</td>
<td>Absence of T Cells, Normal B</td>
</tr>
<tr>
<td>Refractory Anemia with Excess Blasts in Transformation (RAEB-T)</td>
<td>Cell SCID</td>
</tr>
<tr>
<td>Chronic Myelomonocytic Leukemia (JMML)</td>
<td>Common Variable</td>
</tr>
<tr>
<td>Myelodysplastic Syndromes</td>
<td>Immunodeficiency</td>
</tr>
<tr>
<td>Refractory Anemia with Excess Blasts</td>
<td>Wiskott-Aldrich Syndrome</td>
</tr>
<tr>
<td>Refractory Anemia with Ringers Sideroblasts (RARS)</td>
<td>X-Linked</td>
</tr>
<tr>
<td>Refractory Anemia with Excess Blasts in Transformation (RAEB-T)</td>
<td>Lymphoproliferative Disorder</td>
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<tr>
<td>Chronic Myelomonocytic Leukemia (JMML)</td>
<td>Phagocyte Disorders</td>
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<tr>
<td>Stem Cell Disorders</td>
<td>Chediak-Higashi Syndrome</td>
</tr>
<tr>
<td>Aplastic Anemia (Severe)</td>
<td>Chronic Granulomatous Disease</td>
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<td>Fanconi Anemia</td>
<td>Neutrophil Actin</td>
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<tr>
<td>Paroxysmal Nocturnal Hemoglobinuria (PNH)</td>
<td>Reticular Dysgenesis</td>
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<tr>
<td>Pure Red Cell Aplasia</td>
<td>Inherited Erythrocyte Abnormalities</td>
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<tr>
<td>Myeloproliferative Disorders</td>
<td>Beta Thalassemia Major</td>
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<tr>
<td>Acute Myelofibrosis</td>
<td>Sickle Cell Disease</td>
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<tr>
<td>Agnogenic Myeloid</td>
<td>Inherited Metabolic Disorders</td>
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<td>Metaplasia (myelofibrosis)</td>
<td>Mucopolysaccharidoses (MPS)</td>
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<td>Polycythemia Vera</td>
<td>Hurler’s Syndrome (MPS-IH)</td>
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<tr>
<td>Essential Thrombocythemia</td>
<td>Scheie Syndrome (MPS-IS)</td>
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<tr>
<td>Lymphoproliferative Disorders</td>
<td>Other Inherited Disorders</td>
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<td>Non-Hodgkin’s Lymphoma</td>
<td>Hunter’s Syndrome (MPS-II)</td>
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<td>Hodgkin’s Disease</td>
<td>Sanfilippo Syndrome (MPS-III)</td>
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<td></td>
<td>Morquio Syndrome (MPS-IV)</td>
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<td></td>
<td>Maroteaux-Lamy Syndrome (MPS-VI)</td>
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<td></td>
<td>Sly Syndrome, Beta-Glucuronidase Deficiency (MPS-VII)</td>
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<td></td>
<td>Adrenoleukodystrophy</td>
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<td></td>
<td>Mucolipidosis II (I-cell Disease)</td>
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<td></td>
<td>Krabbe Disease</td>
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<td></td>
<td>Gaucher’s Disease</td>
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<td>Niemann-Pick Disease</td>
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<td></td>
<td>Wolman Disease</td>
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<td></td>
<td>Metachromatic Leukodystrophy</td>
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<td></td>
<td>Other Inherited Disorders</td>
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<td></td>
<td>Lesch-Nyhan Syndrome</td>
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<td></td>
<td>Cartilage-Hair Hypoplasia</td>
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<td></td>
<td>Glanzmann</td>
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<td></td>
<td>Thrombocytopenia</td>
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<tr>
<td></td>
<td>Plasma Cell Disorders</td>
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<td></td>
<td>Multiple Myeloma</td>
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<tr>
<td></td>
<td>Plasma Cell Leukemia</td>
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<tr>
<td></td>
<td>Waldenstrom’s</td>
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<tr>
<td></td>
<td>Macroglobulinemia</td>
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<td>Other Malignancies</td>
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<tr>
<td></td>
<td>Breast Cancer</td>
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<tr>
<td></td>
<td>Ewing Sarcoma</td>
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<tr>
<td></td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td></td>
<td>Renal Cell Carcinoma</td>
</tr>
</tbody>
</table>

**THE HLA SYSTEM**

Lymphocytes differentiate between self and non-self cells by examining the HLA antigens expressed on the surface of cells. To prevent graft rejection and other post-transplant complications, stem cell donors and recipients must be closely HLA matched. The HLA antigens are encoded on the short arm of human chromosome 6, on a segment called the major histocompatibility complex (MHC). There are 3.5 million bases in the MHC, but only a small number are currently matched in stem cell transplantation. The relevant portions of the MHC are further divided up into two regions, class I and class II. Class I antigens (HLA-A, -B, and -C) and class II antigens (HLA-DRB1, -DP, and -DQ) are the antigens most frequently examined when matching potential stem cell donors and transplant recipients.

The NMDP requires that donors and patients have no more than one-antigen mismatch at the HLA-A, -B or -DRB1 locations. Because there are two HLA antigens at each of these three locations, a perfect match is referred to as a 6 of 6 match, and a one-antigen mismatch is termed a 5 of 6 match. Using this terminology, the NMDP will allow a 5/6 match or a 6/6 match for marrow and peripheral blood stem cell transplantation.
transplants, but not a 4/6 match or lower. Because of recent research showing better transplant outcomes when HLA-C is also matched, the NMDP now recommends matching at this HLA loci and plans to require HLA-C matching for marrow transplants in mid-2005.7, 8

The NMDP will allow a 4/6 match for cord blood transplants provided, the mismatched antigens. This less stringent HLA matching for cord blood is permitted because of the lowered immunological competence of cord blood T cells.9-11 Some transplant centers require additional matching at the HLA-C, -DP, and/or DQ loci. Newer DNA-based methods of HLA typing have proven to be more accurate than serological methods, and have largely superceded them.12

Impact of HLA match on Transplant Outcome

In allogeneic stem cell transplantation, the degree of donor/recipient HLA match is an important factor in engraftment, the development of graft-versus-host disease (GVHD), and overall survival.13,14 The association of HLA class I allele disparity with graft failure was examined by the Seattle group in which 21 patients experiencing graft failure and 42 case-matched were retrospectively analyzed. Complete allele-level matching for class I was identified in 45 percent of controls and 10 percent of graft failure cases.15 The effect of the number of HLA disparities was subsequently studied in unrelated-donor stem cell transplant for chronic myelogenous leukemia (CML) patients. Among allele-matched transplant and patients mismatched for a single class I allele, the graft failure was 2 percent. When two or more class I disparities at HLA-A, -B, and/or -C was present, the graft failure rate increased to 29 percent.16

In unrelated-donor stem cell transplantation, the risk of clinically significant graft-versus-host disease (GVHD) is also influenced by the extent of HLA disparity between the donor and recipient. GVHD is a potentially life-threatening complication involving an immunological reaction in recipients that is mediated by the transplanted T cells.

SEARCHING THE NMDP REGISTRY FOR AN UNRELATED DONOR

Any physician can submit a preliminary search request free of charge by submitting to the NMDP the patient’s name and address, age, sex, race/ethnic group, disease diagnosis, disease status, and HLA-typing results. The NMDP strongly encourages that patients be HLA typed at A, B, and DRB1 using DNA-based, allele-level typing, but serological HLA typing results are also accepted. The search result will yield more accurate lists of best-matched donors when allele-level tissue typing is performed on the patient. The search process is outlined in Figure 22.2. Because the median time from the start of a search to transplantation is approximately 4 months, physicians are urged to start a search of the NMDP Registry early in the treatment process, even if a stem cell transplant is not part of the immediate treatment plan.

By the next business day following the receipt of a preliminary search request, the NMDP returns a report via fax or mail to the requesting physician showing the potential donors on the NMDP Registry who are 6/6 matches (fully matched at the HLA-A, -B and -DRB1 loci) with the patient. Donors who are 5/6 matches are also shown. Since the NMDP Registry now includes umbilical cord blood (UCB) units stored at NMDP member cord blood banks, the number of matching cord blood units (4/6 matches or higher) will also be reported to the requesting physician.

If the preliminary review of the NMDP Registry reveals one or more potentially matched donors and/or cord blood units; and if the physician and patient decide to pursue a stem cell transplant, a more formal search of the NMDP Registry can be initiated. At this point, charges are assessed to the patient’s transplant center, because a formal search of the NMDP Registry involves additional blood tests of volunteer donors or UCB units. Only physicians at NMDP-affiliated transplant centers can perform formal searches of the NMDP Registry, so the patient must now be referred to an NMDP-affiliated transplant center if he or she is not at one already.

Before a transplant can proceed, a potential donor undergoes confirmatory typing, an additional higher-resolution HLA tissue typing to confirm the results of the original HLA typing. To speed this process, the NMDP uses stored blood samples from volunteer donors whenever possible, but sometimes a fresh blood draw is needed. At this point, a potential marrow or PBSC donor is contacted by phone by NMDP donor center staff to ensure that the donor is in good health and is willing to donate. Obtaining a
confirmatory typing result from a stored cord blood unit requires sending an aliquot from the unit to a central laboratory under contract with the NMDP.

Once the HLA typing of the donor is confirmed, and if the physician and patient decide to proceed to transplant, the NMDP arranges for the donor to be counseled about the risks involved in the donation process. The potential donor receives a physical exam to ensure he/she is medically fit to donate. If the potential donor agrees to donate, the NMDP informs the transplant center and a donation date is scheduled at an NMDP collection or apheresis center closest to where the donor lives. If a UCB unit is selected, the NMDP arranges for the unit to be shipped to the transplant center in a “dry shipper” that keeps the cord blood at the temperature of liquid nitrogen. The average turnaround time from a request to the shipment of a UCB unit is 7 to 10 days.

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Fig. 22.2: The National Marrow Donor Program Search Process. The median time from the start of a search to transplantation is approximately 4 months.
Selection of Donors/Stem Cell Source

For more than 30 years, bone marrow transplantation has been the standard source of stem cells used in transplantation. In the last decade, however, the use of two new sources of stem cells has grown rapidly. There has been a steady increase in the use of allogeneic peripheral blood stem cells (PBSCs) from 20 percent of all allogeneic transplants in 1995 to 55 percent in 2002. The use of cord blood has also risen rapidly. In allogeneic recipients less than 20 years of age without sibling donors, the use of cord blood has increased from <5 percent in 1995 to 17 percent in 2002.17

Stem cells, depending on their source, have different kinetics of hematological recovery and different probabilities of eliciting graft-versus-host disease (GVHD). Engraftment of PBSC grafts is on average faster than engraftment of marrow or cord blood stem cells, although incidence and severity of chronic GVHD is typically greater in PBSC recipients.18,19 Cord blood has generally longer engraftment times, but leads to lower incidence and severity of GVHD.11,20 Transplant physicians must, therefore, consider stem cell source in their donor selection decisions.

Blood Stem Cell Procurement

The NMDP coordinates unrelated donor stem cell transplant using all three sources of stem cells, bone marrow, peripheral blood stem cells and umbilical cord blood. Bone marrow and PBSCs are obtained from our Registry of more than 5.5 million potential volunteer donors. More than 36,000 umbilical cord blood units are available from our growing partnership with cord blood banks.

Marrow Harvest

Bone marrow harvest is one method of obtaining hematopoietic stem cells and is performed at NMDP Collection Centers. It is used less frequently in recent years since PBSCs have become an alternative source of hematopoietic stem cells. Marrow is harvested from the posterior iliac crests while the donor is under general or spinal anesthesia. Large-bore needles are used to aspirate the marrow into syringes that have been rinsed with a heparin-containing solution. The cortical bone is penetrated, and 4 to 10 ml of marrow is aspirated and transferred to a container with anticoagulant solution. The aspiration needle is advanced several millimeters, and the process is repeated. Several aspirations can be made through a single bone puncture, and the bone may be punctured multiple times from a single skin entry site. The amount of marrow collected is determined by the transplant center and is based on the recipient’s weight. However, NMDP regulations prohibit collections > 20 ml/kg of a donor’s weight.

A typical collection involves 200 to 300 marrow aspirations through two skin incisions.21 The volume collected is determined by the nucleated cell count in the collected marrow and by the recipient’s weight. The target is usually 2 to 4 × 10^8 nucleated cells per kilogram of the recipient’s weight.21 When complete, couriers transport the donated marrow stem cells to the recipient’s transplant center.

Marrow harvests are typically very well tolerated by donors, although mild-to-moderate side effects such as localized pain, fatigue and nausea can occur. NMDP data show that overall incidence of serious complications is 1.35 percent. Most serious adverse events are associated either with anesthesia use (39%) or with mechanical injury (59%), i.e., puncture complications caused by marrow collection needles.

PBSC Collection

Many transplant centers have turned to peripheral blood stem cells (PBSC) as the primary source of stem cells for allogeneic transplants. A primary advantage of PBSC is its rapid and durable trilineage engraftment. Although PBSCs are capable of hematopoiesis, peripheral blood in its steady-state does not contain adequate numbers of stem cells to allow for efficient collection. To collect an adequate number of stem cells in the least number of apheresis sessions, it is necessary to stimulate the production of PBSCs through mobilization.

Unrelated PBSC collection must be performed using regimens that pose the least possible risk to the donor yet provide the highest quality graft to the recipient. Mobilization of unrelated volunteer donors is performed using a granulocyte-colony stimulating factor (G-CSF). Most healthy people can tolerate G-CSF administered daily at 5 to 10 μg/kg of donor weight for five to six days; higher doses do not reliably increase the number of CD34+ cells and can result in
more severe side effects.\textsuperscript{21} Apheresis typically starts on day four or five with a second collection on day six if the first collection is inadequate.

The number of circulating CD34+ progenitor cells usually peaks on day five or six of G-CSF injections.\textsuperscript{22} The extent of stem cells mobilized and time of peak mobilization can vary widely among individuals; however, sufficient numbers can reliably be collected from most donors by administering a single daily dose of G-CSF at 7 to 10 μg/kg subcutaneously for four to six days followed by one to three apheresis procedures starting on day three to five.\textsuperscript{23}

Confer et al\textsuperscript{24} reported the NMDP experience of the collection of G-CSF mobilized PBSC from 395 unrelated donors. Adverse reactions to G-CSF included bone pain reported by 85 percent, insomnia (5.8%), headache (5.5%), and malaise (2.5%). Bone pain is effectively treated with over-the-counter analgesics and all other symptoms resolved within a week of cessation of the G-CSF. The NMDP PBSC collection protocol is performed under an IND application with the FDA. This enables the NMDP to collect data about the effects of apheresis, the short-term and long-term effects of G-CSF administration in normal donors, and the efficacy and complications of PBSC transplantation in recipients.

The PBSC are collected by mononuclear cell leukopheresis, usually using a peripheral intravenous catheter. During a single three-hour apheresis procedure, approximately 12 liters of whole blood can be processed. The PBSC collection bag is labeled and taken to the Apheresis Center processing laboratory where the CD34+ cell count is obtained. The usual target for transplantation is 4 to 6 × 10\textsuperscript{6} CD34+ cells per kilogram of the transplant recipient’s weight.\textsuperscript{21} A courier transports the cells from the processing laboratory to the recipient’s transplant center.

\textit{Cord Blood}

Umbilical cord blood (UCB) contains cells capable of hematopoiesis. The first successful UCB transplant was performed in 1988. The early success of UCB transplant and the ongoing need for suitable HLA-matched unrelated donors lead to the establishment of UCB banks in 1993. The NMDP has more than 25,000 UCB units listed on its Registry (November 2002 data). The NMDP cord blood protocol is performed under an IND from the FDA. The advantages of using unrelated UCB include rapid availability, absence of donor risk, lower numbers of GVHD-producing lymphocytes, and lower risk of transmitting infectious diseases such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV).\textsuperscript{25} Disadvantages include fewer stem cells in a typical UCB unit, making this stem cell source less viable for larger children and adult patients. In addition, the original donor is unavailable should a second donation be required due to graft failure or relapse.

Transportation of a UCB unit to a recipient involves transferring a cord blood unit from an NMDP-affiliated cord bank using a “dry shipper” device whose interior is –80°C or colder.

\textbf{Unrelated Donor Stem Cell Transplantation Outcomes}

In the NMDP’s analyses of transplant outcome, the following factors have been shown to affect the overall likelihood of recipient survival as well as other outcome measures such as acute and chronic GVHD and engraftment: diagnosis and stage of disease, degree of HLA match, age of donor, age of recipient, time from diagnosis to transplant, and prior CMV exposure.

The interaction of these factors is complex and varies by diagnosis. In general, improved outcomes are associated with shorter disease duration, younger age of both recipient and donor, six-antigen HLA match, and recipient CMV seronegativity. A representative sample of outcomes of NMDP-facilitated transplants are shown in Figures 22.3 to 22.8.

\textbf{Post-transplant Follow-up}

The NMDP remains involved with unrelated donor transplant recipients following the transplant. The NMDP Research Program collects and studies data on transplant recipients’ successes and complications to learn how to improve patient outcomes. The NMDP collects data on each transplant patient: on the day of transplant, 100 days post-transplant, 180 days post-transplant, and annually thereafter. These data are very important in our effort to continue to learn more about avoiding and treating serious complications and improving transplant outcomes.
Donor follow-up is equally thorough and is coordinated by the NMDP department of Donor Advocacy. The department monitors donor recovery via a donor adverse events medical review team that meets bi-weekly to review both new and long-term cases of donor adverse events.

In the United States, there are more than 20,000 stem cell transplant patients who are more than five years post-transplant (related and unrelated donors). Follow-up for these patients takes place most often in the community setting. It is important that transplant centers educate patients, families and community physicians about the potential for further complications. Close follow-up and rapid intervention when needed provide these patients with the best chance for long-term survival.

**SUMMARY**

Since its founding in 1986, the NMDP has facilitated nearly 20,000 transplants for patients with blood disorders such as leukemia and aplastic anemia, as well as immune system disorders and genetic
disorders. The donors who provided the blood stem cells for these transplants came from the NMDP Registry, the world’s largest and most diverse registry of potential blood stem cell donors. Searching the NMDP Registry for an HLA-matched unrelated donor is as simple as submitting to the NMDP a patient’s HLA typing results along with basic demographic and disease information.

If a suitable donor is identified, the NMDP coordinates the steps needed to bring the donated stem cells—whether they be marrow, PBSC, or cord blood—to a patient. This article is an attempt to bring about a greater understanding among physicians of the complicated process needed to bring about an unrelated donor stem cell transplant. A better understanding of the role the NMDP plays in unrelated donor stem cell transplantation reduces the chances that physicians and patients will have unrealistic expectations about the process.

To initiate a preliminary search of the NMDP Registry, contact the NMDP Office of Patient Advocacy toll-free at 1-888-999-6743. Outside of the United States and Canada, phone 001-612-627-5800. A preliminary search request form can also be submitted via the NMDP Web site: www.marrow.org

REFERENCES


17. Data from the International Bone Marrow Transplant Registry (IBMTR), www.ibmtr.org
Over the past decade, umbilical cord blood banking has evolved into an established, well-rounded program, and the experience with this technology is continuously increasing. Presently, there are approximately 110,000 units of cord blood stored worldwide, approximately 60,000 of these in the United States. In addition to these units donated for unrelated allogeneic transplantation, additional units have been collected by for-profit cord blood banks (CBBs) and are being held for personal use by the donor family. This chapter will provide an overview of the current generally accepted cord blood banking processes.

DONOR RECRUITMENT

Donor recruitment begins in the prenatal period through the obstetric clinic where a nurse or CBB staff person is available during regular business hours to answer related questions. The physicians and their office staff are trained in general criteria for cord blood donation, so they can help guide the mother. For mothers who are not aware of cord blood donation when they arrive at the hospital following the onset of labor, the cord blood program should also be able to provide recruitment materials in the labor and delivery suite. Previously, it was believed that recruitment should not occur while the mother is in active labor. In our experience, while prenatal education is preferred, recruitment in the early stages of labor is acceptable to the majority of mothers. This chapter focuses on the operation of CBBs that collect units for general community use.

OBTAINING CONSENT

While placental blood is usually considered medical waste, obtaining consent for its donation is widely practiced. Maternal and cord blood infectious disease testing requires informed consent. Present guidelines require explicit consent to donation by one or both parents. Consent is generally obtained from the mother because of her availability and of her direct involvement in the infectious disease screening process. If the father objects to the donation, the CBB staff may suggest against the donation.

CBBs differ on how and when consent is obtained. The reasons for this variance include whether the cord blood is collected while the placenta is ex utero or in utero and whether trained staff is available to collect the cord blood during or after the delivery. Most banks, however, obtain consent during the prenatal period.

Consent in the prenatal period is preferable, and may be performed either by trained CBB staff or by patient’s health care provider/clinic staff.

Consent during labor is preferable when dedicated CBB staff performs the collections only during certain days of the week or hours of the day. It prevents disappointment of expectant mothers who are previously consented and cannot donate due to scheduling/staffing constraints. We have established criteria defining mothers who can be approached for intra-labor consent (Table 23.1). The hospital medical staff makes the final judgment regarding the appropriateness of approaching the mother while in labor.
Most organizational policies, including the Guidelines of the New York State Council on Human Blood and Transfusion Services and the Foundation for the Accreditation of Hematopoietic Cell Therapy, do not prohibit consent to collection and donation during labor, and require pre-collection consent for in utero collection.

**Post-collection consent:** Where most organizational policies distinguish between in utero and ex utero collection methods and permit post-collection consent for the ex utero method, the American Academy of Pediatrics strongly opposes post-collection consent for both methods of collection. According to them, “the practice of collecting cord blood first and obtaining permission afterward is considered unethical and should be discouraged.” The AABB standards that require “consent prior to donation” are unclear if that allows consent after blood collection, but before placing the unit into the usable inventory.

**Phased consent process:** Our CBB (American Red Cross North Central Blood Services Cord Blood Bank, St. Paul, MN) employs two methods for obtaining informed consent: (i) obtained in the pre-natal phase, and (ii) obtained during early labor. Hospitals that rely on the patient’s physician/nurse/midwife to collect the cord blood while the placenta is in utero utilize the prenatal consent obtained by the CBB staff since collections can occur whenever the child is delivered. The intra-labor consent is utilized when dedicated Red Cross staff is the collector, but is not available 24 hours/day to perform the collection.

**Prenatal phase:** In the third trimester of pregnancy, the mother is given information about cord blood donation program. If she is interested, then the information is passed to the CBB staff that then obtain maternal consent over telephone and also obtain some general health history information that may preclude the donation. She is asked to re-confirm her consent when she is admitted to the hospital in labor.

**Intra-labor phase:** When a prospective donor/mother is admitted to the hospital in labor, and no previous consent has been obtained, it is possible to approach her for a first-phased consent if her physical condition and labor status meets certain conditions (Table 23.1). When the consent process is initiated while a mother is in labor, an abbreviated, preliminary consent is utilized as a prelude to having the full informed consent obtained, after delivery. The preliminary consent utilized during labor seeks permission to collect and temporarily store the umbilical cord blood until full consent is given. After delivery when the mother is more comfortable, the full consent is obtained.

### MEDICAL EVALUATION OF THE DONOR

The donor screening process, like the process of informed consent, has important ethical and practical issues including what the source should be, what questions to ask, and what to use as an information source. Moreover, while the process of medical evaluation of the donor must assess the infectious and genetic disease transmission risk, it must also produce a product that would be expected to provide satisfactory transplant outcome in terms of engraftment and patient survival. Some of the practical issues related to the donor screening process are discussed below.

#### Medical History Sources

From a medical perspective, an ideal cord blood donor screening process would require that personal and family medical history be obtained from both the parents, in conjunction with a follow-up of infant’s medical status. While this may appear as a justified minimum requirement, this may not be practical or necessary. It may be most intrusive on the family’s time and privacy especially if follow-up medical history is obtained on the infant. Also, it may not be cost effective for most CBBs worldwide. Thus, most CBBs obtain a medical history from the mother including any paternal history that may impact the quality of the cord blood unit. Maternal health is directly linked to the safety of the cord blood.
Obtaining maternal health history along with infectious disease testing is essential and is part of the cord blood banking practice worldwide.

The paternal health history is less directly linked with the health of the infant. It is desirable but is not currently required for banking of a cord blood unit. Until recently, we routinely obtained paternal health history whenever possible. However, less than 1 percent of cord blood units (3 of 301) with available paternal health history were discarded based exclusively on the information provided by the fathers on their health history questionnaires. It was concluded that obtaining paternal history—a logistically difficult and expensive procedure—does not appear to be justified in minimizing infectious disease transmission risk from umbilical cord blood.

The health of the infant is a good indicator of the quantity and quality of cord blood collected. A birth weight of greater than 2,500 grams, a five-minute APGAR score of greater than eight, lack of significant resuscitation efforts for the infant, the newborn exam demonstrating lack of signs for infection and birth defects, are determinants of umbilical cord blood suitability. Bigger babies have been shown to have superior cell counts, CD34+ counts, and CFU-GM; each 500-gram increase in birth weight contributes to a 28 percent increase in CD34+ count. A follow-up of the state-mandated newborn metabolic screen should also be reviewed with particular attention paid to the hemoglobinopathy screening. Many banks will store units that are positive for sickle trait since these units may have HLA types needed for transplantation to certain minority groups. However, it is recommended to perform hemoglobin electrophoresis to rule out compound heterozygotes for hemoglobinopathies. It is also important to notify the transplant center physician, so that the best possible choice of cord units may be made for the patient.

**Medical History Issues**

The maternal and/or paternal history should include the universal blood donor health history questions, and should also obtain information about personal and family history for genetic diseases, and history of any benign or malignant hematological conditions.

The pregnancy and labor history must first be evaluated to determine if a mother and infant should be considered for cord blood donation. The donation process should not put the mother or baby at increased risk. The maternal history also needs to be reviewed to minimize the infectious disease transmission risk to fetus, and to ensure the adequacy of hematopoietic cells in the product to sustain engraftment. Therefore, we recommend the maternal eligibility criteria for referral for ex utero cord blood collection shown in Table 23.1.

Gestational age, gravida status and infant birth weight may affect the number of cord blood stem cells collected. Women with fewer previous live births produce cord blood units with higher cell counts, CD34+ counts, and CFU-GM; each previous birth contributes to a 17 percent decrease in CD34+ count. Babies of longer gestational age have higher cell counts, but lower CD34+ counts and CFU-GM; each week of gestation contributes to a 9 percent decrease in CD34+ cell count. However, it is not practical to exclude collections based on these variables. A more common sense approach is to measure the number of nucleated cells collected to determine which units should be banked.

In our study of 2,084 cord blood units, we found that placental weight >500 grams and meconium in amniotic fluid correlated with better volume, TNC and CD34+ counts, >40 weeks’ gestation predicted enhanced volume and TNC count. Cesarean section, 2- versus 1-person collection and ≤5 minutes between placental delivery and collection produced superior volume. Increased TNC count was also seen in Caucasians, primigravida, female newborns and greater than five-minute collection duration. Ten minutes or fewer minutes between delivery of newborn and placenta predicted better volume and CD34+ count. By regression analysis, collection within five minutes of placental delivery produced superior volume and TNC count. We conclude that donor selection and collection technique modifications may improve product quality. TNC count appears to be more affected by different variables than CD34+ count.

**Obtaining the Medical History**

The maternal medical history may be obtained in the prenatal period postpartum. Obtaining medical history in the antenatal period may be unproductive if cord blood is not collected. On the other hand, when
the medical history is found to be high-risk after the
collection of cord blood, the resources spent in the
collection process are wasted.\textsuperscript{18} It would, therefore,
be desirable for any CBB to obtain donor medical
history at a stage that is most convenient and cost
effective, although this must be done no later than 48
hours after delivery.\textsuperscript{19}

We obtain maternal history in the postpartum
period after the cord blood has been collected except
in rare instances when the mother requests to do it
after the intra-labor consent. When a phased consent
is used,\textsuperscript{8} these questionnaires are typically completed
along with the postpartum consent form for cord
blood storage for possible transplantation, after
collection of the cord blood. The parents may choose
to complete the health history questionnaires prior to
the delivery of the baby. Based on the pregnancy and
delivery records, the information from the health
history questionnaires and the newborn history, it is
decided whether the cord blood unit should be banked
or discarded.

Most CBBs that operate an \textit{ex utero} cord blood
collection program have trained cord blood bank staff
that obtains the medical history and other required
donor information. The cord blood banks that run an
\textit{in utero} collection program may find it more
convenient to involve the obstetric staff in the donor
health history acquisition process, although this is
rarely done because obstetrical staff may not be aware
of the criteria for suitability. Whether it is the dedicated
CBB staff or obstetric staff collecting donor health
history information, it is imperative that the involved
staff be trained to obtain information pertinent to
donor suitability and factors affecting cord blood
quality.

**INFECTIOUS DISEASE SCREENING**

The laboratory tests for infectious diseases are the
same as that presently performed for blood donors.
These are: anti-HIV, HCV RNA, anti-HCV, HCV RNA,
syphilis, anti-HTLV I/II, anti-HB core and HBsAg.
ALT testing is not recommended.\textsuperscript{19} The testing should
be carried out on a maternal peripheral blood sample
in order to conserve the volume of the collected cord
blood unit and provide the most accurate and mean-
ingful results.

Retesting the donor for markers of viral infections
at 6 to 12 months of age to exclude serological window
period infections has been considered in areas endemic
for HIV infections,\textsuperscript{20} but this has not become a
widespread practice. Re-examination of infants for
genetic diseases not apparent at birth has also been
recommended but raises ethical concerns about
parental and infant privacy and might be logistically
difficult as some donors are lost to follow-up. While
controversial, genetic disease testing of the cord blood
may be performed for selected diseases,\textsuperscript{21} if needed.

The medical history evaluation is performed to
make the cord blood as safe as possible for trans-
plantation. The cord blood bank physician frequently
needs to balance whether a donor or family history of
illness will affect the quality of the cord for transplant.
For example, if there is a history of hematologic
disorder that is likely to transmit with the transplant,
the cord should be discarded and not placed in the
useable inventory. On the other hand, it is important
not to exclude cords with a family history of a disorder
not transplanted by hematopoietic stem cells,\textsuperscript{21} lest
there be too few cords available in the bank to meet
the HLA diversity required to maximize the likelihood
of finding a match for a patient in need. CBBs need to
make informed decision on which cords to bank based
sound medical evidence and judgment as part of the
overall quality plan for the program.

**COLLECTION**

In our program, a 250 ml bag (Baxter Healthcare
Corporation, Deerfield, IL) is used. The bag contains
35 ml of CPD anticoagulant. ACD is also accep-
table.\textsuperscript{22,23} The 150-ml collection bag (MedSep Corpo-
ration, Covina, CA) may also be used, but the maxi-
imum collection volume limits some of the larger
collections. It is important when performing an \textit{in utero}
collection to make the physician aware that the
bag is not sterile and, therefore, should not be placed
on a sterile field.

Samples of the cord blood product can be removed
from the tubing for HLA and ABO/Rh testing and
retention. The cord blood unit is then stored and
shipped on wet ice for transport to the processing site.
We recommend that cryopreservation of the cord
blood unit be completed within 48 hours of collection
Umbilical Cord Blood Banking

In Utero Collection Method

In utero cord blood collection is performed in the delivery room by the patient’s physician/midwife, after the umbilical cord has been clamped and cut. The collection bag containing anticoagulant is prepared by placing a hemostat on the tubing a few inches from the needle. The umbilical cord is then cleansed by wiping the intended insertion site with sterile gauze and a 30-second betadine scrub is performed. The needle attached to the blood bag is inserted into the umbilical vein. Once the hemostat is removed, the blood flows into the bag by gravity. It is important to remember not to remove the hemostat prior to insertion of the needle lest room air contaminate the bag. During the 5 to 8 minutes required to collect the blood from the cord and placenta, the collection bag should be gently rotated to mix the blood with the anticoagulant. The collection is complete when blood ceases entering the bag. It may be helpful to place the collection bag on a scale to monitor blood flow and determine when it has stopped. The blood in the tubing is then “stripped” into the collection bag to mix with the anticoagulant.

Ex utero Collection Method

Since ex utero collections can be performed outside of the delivery room, a trained cord blood collection technician can perform the collection. Trained staff have fewer instances of bacterial contamination and labeling errors. In the delivery room, following delivery, the physician/nurse/midwife clamps and cuts the cord. If possible, within 30 seconds of the birth, the cord should be double clamped 3 to 5 cm from the baby’s umbilicus. The optimal interval of time between clamping and cutting of the cord should be less than 5 seconds. The placenta and cord are taken to a nearby room where the placenta is suspended in some manner to allow gravity to increase the blood flow. Our program uses a specially designed framework welded out of metal that holds a 12-cup plastic bowl approximately 3 feet above the work surface. The bowl has a 2 cm sized hole drilled in it for the cord to be threaded through. Prior to collection, the bowl is lined with a disposable paper towel to absorb the excess external placental blood, thereby prohibiting it from dripping down the cleansed cord.

Cord preparation and collection are performed in the same manner as in the in utero method. Additional manipulation of the cord can be performed, such as “milking” the cord between 2 fingers toward the placenta, or holding the distal end of the cord above the needle insertion site for the aid of gravity. A well-trained person can perform these tasks independently. While the presence of a second staff person does increase the volume collected, it does not affect the number of stem cells collected and adds to the cost of the units collected.

Liquid Preservation of Cord Blood

Several studies of the short-term liquid storage of hematopoietic stem cells obtained from marrow and peripheral blood have established that tissue culture solutions with anticoagulants or anticoagulated plasma can satisfactorily preserve stem cells at 4°C or room temperature for 24 hours or more. The successful transplantation of marrow stored in the liquid state over several days demonstrates that such storage can be successful. Despite this experience, there are no generally accepted guidelines or criteria for neither short-term liquid storage of hematopoietic stem cells nor are there any preservative solutions FDA licensed for this purpose. Even fewer data are available for cord blood.

Shlebak and colleagues found that a significant reduction in frequency of CFU-GM for undiluted cord blood stored for 9 hours or more at either 4 or 25°C and similar results were reported by Ademokun et al. Campos et al observed > 95 percent recovery of TNC and CFU-GM for cells preserved at room temperature for 24 hours and then cryopreserved. Storage at 4°C resulted in more rapid degradation. These contradictory results and the limited amount of data indicate that further study of the optimum short-term storage conditions of cord blood is warranted.

Hubel and associates compared the recovery and viability of cord blood cells stored in different solutions. The use of a short-term storage solution for the liquid storage of cord blood improves the retention of viable cells compared with storage in CPD anticoagulant. Cord blood can be stored for at least 24 hours before an important loss in the number of
mononuclear cells, CD34+ cells or CFU-GM occurs. Storage at 4°C may be preferable to room temperature. Of the preservation solutions tested in this study, Normosol, Plasmalyte A and STM-Sav were found to be satisfactory. It was recommended that cord blood could be stored in any of these three solutions for up to 48 hours at 4°C before processing.

PROCESSING, CRYOPRESERVATION AND STORAGE

Shipment to Processing Facility

Although information about cord blood preservation is becoming available, there is no uniformly accepted storage method or time limit. Thus, cord blood products may be transported for processing at room temperature, on ice or with insulated pre-cooled stabilizing packs.

A primary, outer container that is leak-proof, constructed to resist breakage, and durable enough to withstand pressure changes and falls must be used. An inner, secondary container such as a plastic, re-sealable bag with enough absorbent material to contain the contents of the product in the event of a leak or break must also be used. Information with the name, address and phone number of the shipping and receiving facility must be on the outside and inside of the primary shipping container. Additional labeling requirements such as: biohazard labels (as appropriate) and instructions not to expose package to radiation are also needed. Units should be processed and cryopreserved within 48 hours of collection. Logistically, this may lead to problems with units received from distant sites. More frequent shipping and same day shipping may be necessary.

Receipt at Processing Facility

Upon arrival in the cord blood processing laboratory, documentation of receipt and visual inspection of the product for blood clots/discoloration/bag leaks are performed. A log may be used to capture the time the product was received, and the temperature upon receipt. Appropriate labeling must be confirmed.

Initial Processing Criteria

Many unrelated cord blood banks have established minimum total nucleated and volume pre-processing criteria in an effort to improve the quality of units banked. We recommend a minimum of 50 ml cord blood or $10 \times 10^8$ total nucleated cells. Units not meeting these criteria can be discarded, used for quality assurance, or research. In our experience with cord blood units collected by either obstetricians or dedicated, trained collection staff, approximately 50 percent of all units received are processed and frozen.

Processing, Cryopreservation and Storage

When determining the processing, cryopreservation and storage methods for a CBB many factors must be considered. First and foremost is to establish a method that ensures the function, integrity and safety of the product. Defined workspace and processing conditions (e.g. one cord blood unit per technologist at a given time or one product in the field during product labeling or transfer) are safeguards used by processing laboratories. Using a closed bag system minimizes the risk for contamination and assists in preserving product identity.

Current practice has been to minimize the amount of red blood cells and reduce the volume of the freezable product. This reduces the potential adverse effects of incompatible and lyzed hemolysate and maximizes storage capacity. There are many methods to reduce the volume of red blood cells and plasma with the addition of various media to aid in separation such as gelatin, ficoll, percoll, hydroxyethyl starch, dextran, and poligeline.

Most laboratories process cord blood based on the method developed by Rubinstein, which includes the depletion of red blood cells using hydroxyethyl starch (HES) followed by a leukocyte concentration step. This method optimizes storage space and minimizes the potential of infusion-related complications associated with large volumes of dimethylsulfoxide (DMSO) and red blood cells. We use a slightly modified version of the Rubinstein method.

Prior to the addition of the HES, the initial cord blood unit is weighed and a product volume is determined. The specific gravity of whole blood 1.06 g/ml may be used for the conversion from grams to milliliters. Cord blood units for allogeneic unrelated and related use must be typed for HLA class I and class II antigens including HLA-A, B and DRB1 loci. Pre-cryopreservation samples are taken for nucleated cell count, differential count, clonogenic colony-forming unit (CFU) cell assay, CD34+ analysis,
sterility (aerobic, anaerobic and fungal) cultures, nucleated cell viability, and ABO blood group and Rh type. We freeze an integrally attached, labeled segment along with two additional detached and labeled segments with each cord blood unit. These segments can be used for HLA confirmatory typing and/or identity, ABO/Rh identity, viability, CD34+ analysis, progenitor assays or other tests that may be useful prior to releasing the cord blood unit to a transplant facility. Tests to confirm the identity of the cord blood unit are essential to avoid transplanting a mislabeled unit. In accordance with FACT standards, two vials of the leukocyte poor plasma are frozen and two vials of 1 to 2 $\times 10^6$ mononuclear cells and 50 micrograms of genomic DNA are frozen and stored.

Once the red cell depletion and volume reduction process has been performed and all samples have been removed, the product volume is generally between 20 and 25 ml. Cryopreservation is then performed by adding a cryoprotectant solution of dimethylsulfoxide (DMSO) and dextran 40 to the red cell depleted cord blood unit. The final DMSO and dextran concentrations are 10 percent and 1 percent, respectively. The product is frozen in a cryogenic bag in a controlled rate freezer. After freezing, products are transferred to continuously monitored, long term, liquid nitrogen storage containers (<–135°C). Products are stored in the vapor phase of liquid nitrogen to minimize the potential for cross contamination. The liquid phase of LN2 or mechanical freezers may be used as well. The allowable length of storage is currently undefined. However, studies have shown that frozen cord blood cells can be stored safely for prolonged intervals (i.e. ten years) without substantial loss in hematopoietic progenitor cells.

All processing documents must be maintained, and reviewed by trained personnel. Completed documentation should also be reviewed for quality assurance. Moreover, a cord blood bank requires various laboratory quality control systems to substantiate the integrity and quality of the cord blood units banked.

**TRANSPORTATION OF CORD BLOOD FOR TRANSPLANTATION**

Depending upon the urgency in which the product is needed, notification of a request for shipment of a cord unit may be more than two weeks or as little as 12 hours. Cord blood units are shipped in LN2 dry shippers that have been “charged” 24 hours prior to release. Dry shippers are insulated containers that have a shell that absorbs liquid nitrogen and creates an environment that is similar to the vapor phase of an LN2 vapor tank. The temperature must remain below –135°C during shipment. When a dry shipper is “charged” properly, the shipper will maintain the maximum acceptable performance and temperature. To be considered acceptable, the shipper must maintain temperature at least 48 hours beyond the expected time of arrival at the receiving facility. We have found the dry shippers to maintain a constant temperature of <–150°C for at least eight days. If a continuous monitor is not used, at a minimum, a temperature indicator must be included in all cryogenic shipping containers to indicate whether an established temperature range has been exceeded.

As with cord blood products that are shipped prior to processing, adherence to transportation regulations by regional blood bank associations/governmental bodies is required.

For cross country or international shipment of containers exposed to standard airport security X-ray machine, National Marrow Donor Program (NMDP) Donor and Safety Monitoring Committee has determined that based on the doses of X-rays delivered, the risk to HSC products was nil.

**THAWING AND WASHING OF UMBILICAL CORD BLOOD UNITS**

The cord blood thawing procedure is similar to that of other hematopoietic stem cells (HSCs). The unit should be carefully removed from the storage container, and an inspection to evaluate the integrity of the bag should be performed. Following label verification of product identity by two technologists, the unit is placed and tightly sealed within a clean or sterile “zip-lock” bag and submerged in a 37°C water bath. The thaw should be performed relatively quickly to prevent recrystallization and consequent cell damage/death. Gentle kneading of the contents will help to accelerate the process. If a leak is discovered, the site of the break should be determined, and a hemostat should be used to prevent further escape of the product. The contents of a broken or leaking bag should be aseptically transferred into a transfer bag under a biological safety cabinet.

The cord blood wash procedure is based on the processing and cryopreservation methodology originally described by Rubinstein et al. Between
institutions, there may be slight modifications of this simple procedure with regard to centrifugation (i.e., rpm, duration of spin, etc.), concentration of solutions used for dilution/resuspension, etc. For details of the wash procedure in use at Fairview-University of Minnesota Medical Center, please refer to reference.\(^1\) The final product has a volume of approximately either 60 ml or 100 ml. Samples are removed for cell counts (TNC, CD34+, and CD3+), culture (bacterial, yeast), confirmatory ABO/Rh typing, and nucleated cell viability testing. Final volume, dose, and recovery are determined. After the necessary forms and labels are completed, the cord blood can be released for delivery to the patient care unit. Although there is little data available,\(^46\) we recommend that the thawed/washed cord blood be transfused as soon as possible.

**TRANSFUSION OF CORD BLOOD**

We recommend reviewing the general process, potential side effects/adverse reactions, and plans for premedication to avoid or alleviate any side effects/reactions, and the overall expectations with the patient.

**Side Effects and Adverse Reactions and Premedication**

Reactions similar to those seen with blood transfusion (i.e., allergic, hemolytic, febrile, and those due to microbial contamination) may be seen with the infusion of HSCs. However, because of current standard cord blood processing and thawing methods, which include steps for red cell depletion, removal of excess plasma, and a post-thaw wash, reactions typically attributed to red cells and plasma proteins (i.e. cytokines, antibodies, etc.) are not nearly as common in the setting of cord blood infusion.

The post-thaw wash essentially eliminates the minimal risk of renal failure and other reactions due to infusion of small amounts of cellular debris (e.g. red cells and granulocytes) and free hemoglobin, products of cell lysis from the freezing process. Reactions classically attributed to DMSO ranging from mild nausea, vomiting, cough, and headache to cardiac arrhythmia and cardiopulmonary arrest\(^40,47,48\) are also less common. It remains to be determined, however, if the reduction of DMSO-related reactions is truly attributable to the wash step, as the amount of DMSO prior to washing is minimal (less than 1 mg/kg in even the smaller patients). Studies to evaluate the need for the wash step, weighing the questionable benefits of DMSO removal against the consequent loss of HSCs, are underway.

Bacterial contamination remains at least a minor problem in cord blood banking with rates at collection approaching 5 percent.\(^24,49,50\) One report suggests slightly higher rates of contamination with *in utero* collection.\(^24\) Some banks have retained contaminated units (depending on the organism), notifying interested transplant centers of the identity of the organism and its susceptibility to antibiotics. Most banks, however, discard contaminated units, avoiding any future concerns upon infusion. If a unit contaminated at collection or at some point in processing or thawing is infused, however, general use of broad-spectrum antibiotics in this patient population fortunately makes adverse reactions secondary to infusion of contaminated cord blood units uncommon as well.

A review of all cord blood transplants (59 patients/82 units) performed at Fairview-University of Minnesota Medical Center during 2002 showed no associated severe adverse reactions or complications associated with cord blood infusion.\(^51\) Thirty-one of fifty one (61%) patients from whom data was available had no reaction/complication associated with infusion. The remaining 39 percent experienced or reported one or more of the following: transient increase in blood pressure (16%), nausea (8%), episode(s) of vomiting (4%), unusual taste/smell (6%), mild bradycardia (2%), mild, transient cough (2%), non-symptomatic oxygen desaturation (2%), back pain (5%) or abdominal pain (2%) responsive to pain medication. No patient experienced fever/chills, hives, hypotension, dyspnea, bronchospasm, or chest pain. Although severe cord blood infusion reactions are rare, the possibility for such a reaction to occur remains. Therefore, aggressive intravenous (IV) hydration (e.g. 2–6 hours before and 6 hours after infusion, with diuretics as needed) should be standard procedure, and general use of prophylactic antiemetics, antipyretics, and antihistamines is recommended.

**Administration of Cord Blood Units**

Once the cord blood unit is thawed and washed, it is delivered to the patient care unit without delay. The nursing staff signs a release form acknowledging receipt of the unit and notifies the patient’s physician
of its arrival. Following physician approval for infusion and proper identification procedures, the unit is infused by IV drip directly into a central line without a needle, pump, or filter. Because the thaw/wash procedure is roughly two to three hours in duration and prolonged exposure to DMSO is thought to be toxic to cells, cord blood should be infused as quickly as tolerated. Although very little DMSO is present in the unit (the intracellular portion that remained during the wash), to assure optimal viability the infusion should ideally be completed within 15 to 30 minutes of its receipt on the floor. Because the total volume is 60 to 100 ml, completion of infusion in this timeframe is generally easily accomplished.

Although some institutions use a standard blood administration filter (170 micron) to capture clots/aggregates during infusion of marrow and peripheral blood stem cells, such filters are not used by all transplant centers when cord blood is the source of HSCs. Sterile saline may be added directly to the unit bag in an attempt to increase flow rate if the flow becomes unusually slow. In all cases, the nursing staff should be instructed to flush the unit bag and IV tubing with sterile saline after the unit bag empties to minimize cell loss and, therefore, maximize cell dose.

At a minimum, vital signs should be checked before infusion and immediately and one hour after infusion. If any adverse reactions are associated with the infusion, more frequent vital sign checks may be required. The patient’s physician and the medical director of the cell processing laboratory should be notified immediately of any signs or symptoms of an unexpected or severe adverse reaction. In the event of such an adverse reaction, an investigation, with appropriate laboratory testing (e.g. DAT, antibody titers, gram stain, culture, etc.), similar to that of a suspected blood transfusion reaction should be initiated. An infusion form should accompany the cord blood unit, and its completion should be required. The completed form should contain at a minimum the name and unique identifier of the recipient, the proper product name and product identifier, and the initials of the medical staff receiving the product. We further recommend that the form have space designated for cell dose, unit volume, proper identification procedure, date, start time and duration of infusion, description of patient status prior to and after infusion, and any complications associated with the procedure.

### QUALITY ASSURANCE AND QUALITY CONTROL IN CORD BLOOD BANKING

From collection and processing through transplant and follow-up, a cord blood quality assurance (QA) program establishes a series of controls, quality monitors, and feedback mechanisms that ensure product uniformity while revealing trends, preventing errors, and promoting continuous process improvements.

A well-designed QA program will enable a cord blood bank to maintain current good manufacturing practices (cGMP)/current good tissue practices (cGTP) and, thus, adherence to regulatory requirements. Designing a QA program that is adaptable to the current, evolving nature of regulations/standards is paramount to operating in a compliant and highly controlled environment.

Although GMP requirements are considered more extensive and rigorous than those of GTP, they both share common quality essentials such as: a quality management plan, document and process control, deviation and adverse reaction reporting, facility management, equipment validation, training, donor screening, environmental monitoring, supply management, validation, process control, product labeling, storage, distribution, quality control testing, quality audits, and complaint file.

It is the role of the Quality Assurance unit to oversee the QA program for the cord blood bank. This group generally is responsible for the documentation system, conduction of audits of processes, systems, results and methods, and performance of trend analysis. Depending upon the size of the organization, the QA officer may work within the department or be part of the institution’s overall QA program. The designated QA person, however, should not oversee the work that he or she has performed.

Additionally, Table 23.2 summarizes quality control, an integral part of QA designed to “address the evaluation of raw materials, in-process materials, packaging, labeling, and finished products.”

<table>
<thead>
<tr>
<th>Table 23.2: Recommended quality control testing</th>
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<tr>
<td><strong>Postprocessing-precryopreservation testing</strong></td>
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<tr>
<td>Nucleated cell count, WBC count and differential</td>
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<tr>
<td>Hematocrit, nucleated RBC</td>
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<td>Nucleated cell viability</td>
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<td>CD34</td>
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<td>CFU</td>
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<td>Sterility — aerobic, anaerobic and fungal</td>
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45. McKenna D, Wagner J, McCullough J. Umbilical cord blood infusions are associated with mild reactions and are overall well-tolerated. Presented at the 9th Annual Meeting of the International Society for Cellular Therapy, Phoenix, Arizona (May 29-June 1, 2003).


Hematopoietic stem cell (HSC) transplantation has become an increasingly viable option in the treatment of both malignant and nonmalignant disease. Transplant success relies upon sound, established methods of HSC processing. The goal of this chapter is to provide an overview of HSCs while familiarizing the reader with these methods. The following topics will be discussed: sources of HSCs, processing methods, cryopreservation, quality control testing, quality assurance and regulatory issues.

**SOURCES OF HEMATOPOIETIC STEM CELLS**

Summaries of the three sources of HSCs [bone marrow (BM), peripheral blood (PB), and umbilical cord blood (UCB)], including advantages, etc. are outlined in Table 24.1.

**Bone Marrow**

Bone marrow (BM) has been the traditional source of HSCs. However, use of BM has decreased over recent years, as other sources of HSCs with clear advantages have been found. Despite the decrease in use, a primary role for BM in HSC transplantation remains. The more recent identification of BM as a source rich in mesenchymal stem cells and other multipotent cells\(^1,2\) further indicates the continued utility of BM. Following harvest (10–15 ml/kg recipient weight) at the posterior iliac crest, filtration (typically sequential in-line filters of decreasing filter pore size) makes the collection free of bone spicules and other debris. Target dosage (typically 2–4 × 10\(^8\) nucleated cells/kg recipient body weight) can be efficiently collected in a single procedure.

**Peripheral Blood**

Studies performed several years ago showed HSCs to be in the peripheral blood (PB) at very low concentrations.\(^3\) Later discoveries of hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, or GM-CSF, and granulocyte colony-stimulating factor, or G-CSF) and their role in mobilization of HSCs from BM led to development of apheresis strategies to collect HSCs from the mononuclear cell fraction of blood.\(^4\) Venous access (peripheral or central) is utilized in a procedure that lasts roughly five hours, depending on processing volume. Many centers use rising peripheral white blood cell (WBC) count and/or pre-collection peripheral blood CD34+ cell enumeration (usually 10–12 CD34+ cells/\(\mu l\)) to determine the most efficient collection plan, i.e. date, process volume or length of collection.\(^5,6\) Using this strategy, most healthy G-CSF-stimulated allogeneic donors need one to two collections to reach the target dose. In the autologous setting more collections are typically required, as mobilization may be difficult due to disease, history of chemotherapy and/or radiation, etc.

The same apheresis strategies (often without growth factor stimulation) may be utilized to collect PB mononuclear cells for use as raw material in the production of a variety of other cellular therapies. Complexity of processing of these therapies [e.g. donor lymphocyte infusion (DLI), natural killer (NK) cells, dendritic cells, and antigen (i.e. cytomegalovirus, Epstein-Barr virus, tumor, etc.)-specific T-cell immunotherapies] varies markedly from minimal (quality control testing only) to extensive (cell selec-
Hematopoietic Stem Cell Processing

279

These products may serve as adjunctive or supportive therapies, post-HSC transplant or as therapies altogether independent of HSC transplant.

Umbilical Cord Blood

Once regarded as biological waste, umbilical cord blood (UCB) has been demonstrated to contain HSCs with higher proliferative and self-renewal capacity than those of BM and PB. At most institutions, UCB transplantation is limited to children and small adults because of concerns of appropriateness of HSC dose. However, several strategies have been initiated to overcome limitations of dose. Transplantation of two or more units and ex vivo expansion are two such strategies.

Currently, most collections are by “closed-system” involving umbilical vein cannulation or venipuncture and direct drainage into a plastic blood bag. The unit then proceeds to processing and cryopreservation and is stored in liquid nitrogen for eventual use. For further in-depth discussion of UCB, the reader is referred to Broxmeyer Hal E (Ed): Cord Blood — Biology, Immunology, Banking, and Clinical Transplantation. Bethesda, MD: AABB Press, 2004.

PROCESSING METHODS

HSC processing involves both routine and specialized methods. The routine methods utilize concepts and equipment that are well-established in the blood bank setting. The specialized methods, on the other hand, involve concepts, reagents, and instruments unique to cell processing/engineering.

Routine Methods

Routine methods include volume (plasma) reduction, red cell depletion, buff coat preparation, thawing/washing, and filtration. Volume reduction involves centrifugation. It is performed in the settings of minor ABO mismatched allograft (BM and PB) transplantation to reduce incompatible plasma and in the transplantation of patients with fluid balance/overload issues (e.g. small patients, renal/cardiac failure patients). Volume reduction may also be performed for purposes of cryopreservation (i.e. storage space limitations or manipulation/optimization of cell concentration).

### Table 24.1: Sources of hematopoietic stem cells

<table>
<thead>
<tr>
<th>Source</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow</td>
<td>Traditional source of HSCs; decreasing in use as HSC graft; recently identified as rich source of MSCs, MAPCs</td>
</tr>
<tr>
<td></td>
<td>Used predominantly in allografting (occasional autograft procurement for HSC back-up or if poor mobilization in patient)</td>
</tr>
<tr>
<td></td>
<td>Harvesting procedure to collect 10–15 ml/kg recipient weight (nucleated cell target dose: 2.0–4.0 × 10⁸/kg) requires general anesthesia.</td>
</tr>
<tr>
<td></td>
<td>Advantages: only one procedure needed for full collection; relatively lower T-cell content</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>Common place in the autologous setting; replacing bone marrow as the primary allogeneic HSC source.</td>
</tr>
<tr>
<td></td>
<td>Requires mobilization in the patient/donor with either chemotherapy, hematopoietic growth factors, or both (CD34+ cell target dose: 5.0 × 10⁶/kg)</td>
</tr>
<tr>
<td></td>
<td>Advantages: no need for general anesthesia/hospitalization; rapid engraftment; possibly lower tumor cell contamination (compared to BM) in autologous transplant setting</td>
</tr>
<tr>
<td>Umbilical cord blood</td>
<td>Collected from the umbilical cord and placenta by dedicated staff or obstetrician following delivery (in utero or ex utero)</td>
</tr>
<tr>
<td></td>
<td>Stored either for eventual use by the family or (more commonly) banked for transplant into unrelated patient.</td>
</tr>
<tr>
<td></td>
<td>Minimum CD34+ cell dose: 1.7–2.0 × 10⁹/kg</td>
</tr>
<tr>
<td></td>
<td>Advantages: decreased incidence of graft-versus-host disease; decreased search time; reduced histocompatibility matching requirements</td>
</tr>
</tbody>
</table>

Red blood cell (RBC) depletion employs sedimenting agents (e.g., hydroxyethyl starch) to reduce red cell content. It is used to prevent hemolytic transfusion reactions when major ABO — and other clinically relevant RBC antigen (e.g., Kell, Kidd) — incompatible BM allografts are transplanted. Also, RBC depletion prior to freezing limits the amount of lysed RBC fragments and free hemoglobin upon infusion. RBC depletion may also be useful when storage space is a concern (e.g., UCB banking).

Buffy coat concentration of BM involves centrifugation and harvesting of the white blood cell fraction and can be performed with an apheresis or cell washing device. Manual centrifugation may be used when product volume is too low for apheresis or cell washing devices. Buffy coat preparation is generally used for volume reduction for cryopreservation or as a method of RBC depletion prior to further manipulation.

The thaw procedure for all HSCs, regardless of source, is similar. Although quite simple, proper execution is essential, as frozen plastic containers may be prone to breakage for a variety of reasons. The product should be handled carefully while inspecting to verify identity and ensure integrity of the bag. The product is submerged in a 37°C water bath within a clean or sterile plastic bag. Gentle kneading allows the thaw procedure to proceed relatively quickly preventing recrystallization and consequent cell damage/death. A hemostat should be used to prevent loss of the product if a break is noted; contents should be aseptically diverted into a transfer bag, and a sample should be sent for culture.

Washing of HSCs serves to remove lysed red cells, hemoglobin, and cryoprotectant (dimethylsulfoxide, or DMSO). UCB, despite an RBC depletion step prior to freezing, is the primary product that is routinely washed. Most institutions base their UCB processing methodology, including the thaw/wash procedure, on that originally described by Dr. Pablo Rubinstein of the New York Placental Blood Program. Briefly, the thaw involves slow, sequential addition of a wash solution (e.g., 10% dextran, then 5% albumin), transfer into a bag of appropriate size for centrifugation, and resuspension of cell pellet(s) before delivery to the patient care unit for infusion.

As noted above, BM typically undergoes sequential filtration in the operating room or in the laboratory to remove aggregates/debris. Opinions regarding use of standard blood filters upon infusion of HSCs vary, however. The decision to use a standard blood filter (>170 microns) rests with the individual cell processing laboratory and/or transplant center. If an institution opts to use a standard blood filter, the laboratory should, of course, validate their filtration process.

**Specialized Methods**

In general, specialized cell processing methods optimize product purity and potency beyond levels obtainable by routine methods. Examples of these methods include elutriation, cell selection systems, and cell expansion. While centrifugation separates cells based upon density alone, counterflow centrifugal elutriation allows for cell separation based upon both cell density and size (sedimentation coefficient). Fluid/media is passed through cell layers opposite to a centrifugal field. Adjustment of the flow rate and/or the speed of centrifugation to enable counterflow rate to balance centrifugal force allows for alignment of cells. Cell populations may then be diverted as fractions of the initial product. T-cell depletion has been the primary application of elutriation, but other applications, including monocyte enrichment for dendritic cell generation, exist and are increasing in use.

Cell selection systems incorporating monoclonal antibody-based technologies that target cell surface antigens have increased in popularity. These specialized immunomagnetic methods provide a highly selective means for obtaining specific cell types, attaining an unequaled level of purity. Clinical-grade reagents allow for selection of HSCs (CD34+ or CD133+). Several other clinical-grade reagents have been developed or are in development (e.g., anti-CD3, anti-CD14, anti-CD19, and anti-CD56) and will make selection of B- and T-cells, monocytes, NK cells and other cell types possible as well.

*Ex vivo* expansion of HSCs and progenitors have been a focus for hematology and transplant researchers because of the strong correlation between cell dose (nucleated cell, CD34+ cell, and colony-forming cell) and patient outcome. It is thought that successful expansion would enhance hematopoietic engraftment while reducing transfusion dependence, risk of
infection, and duration of hospitalization, as well as support a variety of clinical applications (e.g. gene therapy, immunotherapy). For a thorough review of the current status of HSC expansion, the reader is referred to Devine SM, Lazarus HM, Emerson SG. Clinical application of hematopoietic progenitor cell expansion: current status and future prospects. Bone Marrow Transplantation (2003); 31: 241-252.

CRYOPRESERVATION

As HSCs may need to be stored for weeks to years prior to transplant, methods for cryopreservation are necessary. Most cell processing laboratories use the cryoprotectant DMSO, commonly at 10 percent concentration, and a source of plasma protein for cryopreservation of HSCs. Some laboratories add hydroxyethyl starch (HES), which allows for a decreased concentration of DMSO (e.g. 5% DMSO/6% HES). DMSO is a colligative cryoprotectant; it diffuses rapidly into the cell, reducing the osmotic stress on the cell membrane. DMSO prevents dehydration injury by moderating the non-penetrating extracellular solutes that increase during ice formation. It also slows extracellular ice crystal formation. HES is a non-penetrating (extracellular), macromolecular cryoprotectant. It is a high-molecular weight polymer that likely protects the cell by forming a glassy shell, or membrane, around the cell, retarding the movement of water out of the cell and into the extracellular ice crystals.

Freezing of HSCs may be at a controlled rate or a non-controlled rate (e.g. simply transferring HSCs into a freezer bag and placing in a –80°C mechanical freezer). Controlled rate freezing utilizes computer programming to incrementally decrease HSC product temperature in a controlled fashion. The initial cooling rate is 1°C/minute, and this proceeds to (liquid to solid) phase change (roughly between –14 and –24°C), at which time a rapid, supercooling occurs. Following warming of the chamber to the end phase change temperature, cooling continues at the rate of 1°C/minute until the product has reached –60°C. At this point, the product is cooled at 3°C/minute until it reaches –100°C. Following both controlled-rate freezing and non-controlled-rate freezing, the HSC product is transferred to a storage freezer. Most laboratories store HSCs in the vapor phase of liquid nitrogen at temperatures below –135°C. The liquid phase of LN₂ or mechanical freezers, though not ideal, may be used as well.

QUALITY CONTROL TESTING TECHNIQUES

Quality control (QC) testing in the clinical cell therapy laboratory serves two purposes — to determine the suitability and safety of the cellular product for the individual patient and to monitor overall laboratory practices. Testing is aimed at characterizing the safety, purity, identity, potency, and stability of the cellular product, and the extent of testing is primarily dependent upon complexity of manufacturing. Testing may include simple tests (e.g. nucleated cell count) and/or complex tests (e.g. molecular or cytogenetic analysis). Common QC tests for HSCs include cell count and differential, viability and colony-forming unit assays, CD34+ cell enumeration, and sterility testing. The CD34+ cell enumeration strategy at the University of Minnesota is based upon the International Society for Hematotherapy and Graft Engineering, or ISHAGE (now the International Society for Cellular Therapy, or ISCT), Guidelines for CD34+ Cell Determination by Flow Cytometry. Table 24.2 summarizes the common QC tests for HSCs.

QUALITY ASSURANCE (QA) AND REGULATORY ISSUES

Quality assurance (QA) per the Food and Drug Administration (USA) is the sum of activities planned and performed to provide confidence that all systems that influence product quality are reliable and functioning as expected. The QA, or quality, program defines the policies and environment necessary to attain minimum quality and safety standards. The basic components of a program include standard operating procedures (SOPs), documentation/recordkeeping and traceability requirements, personnel qualifications and training including a continuing education program, building and facilities/equipment quality control (QC), process control, auditing and investigation, and error and accident system/management. Regulatory authorities worldwide, realizing the importance of QA, have placed major emphasis on the establishment of an effective quality program. It is expected that the HSC collection/processing center’s quality program will ensure a laboratory’s compliance with regulations.
The current worldwide regulatory atmosphere reflects the rapid growth in cellular therapy as it is evolving equally rapidly. Several regulatory initiatives have been underway in Europe; the European Commission (EU) is designing a directive to ensure the quality and safety of human tissues and cells with plans for implementation in 2005–2006. The Joint Accreditation Committee of the International Society for Cellular Therapy Europe (ISCT Europe) and the European Group for Blood and Marrow Transplantation (EBMT), or JACIE, which was created in 1997, has recently published the second edition of the JACIE Standards. The JACIE Standards will likely serve as general guidance for regulatory compliance throughout Europe. In the United Kingdom (UK), specifically, further regulatory guidance is provided in a code of practice for tissue banks providing tissues of human origin for therapeutic purposes and the Guidelines for the blood transfusion services in the United Kingdom, or the Red Book. In the USA, the Food and Drug Administration has established a tiered, risk-based system to regulate cellular therapies. The current good manufacturing practices (cGMP) and current good tissue practices (cGTP) will provide the framework for this system. The Foundation for the Accreditation of Cellular Therapy (FACT) and the American Association of Blood Banks (AABB), both professional organizations, have written standards as guidance toward compliance with the regulations.

ACKNOWLEDGMENT

This chapter was written by shortening and modifying a chapter previously written by the authors and published in: Haemopoietic Stem Cell Processing and Storage, MF Murphy, DH Pamphilon (Eds): Practical Transfusion Medicine, 2nd edn. Blackwell Publishing 2005 Table 4.

Table 24.2: Quality control testing of hematopoietic stem cells

<table>
<thead>
<tr>
<th>Test</th>
<th>Methods</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell counts</td>
<td>Hematology analyzer, manual differential</td>
<td>No hematology analyzer is truly designed for analysis of HSC products. A manual differential may be more helpful in determining mononuclear cell content and HSC quantity.</td>
</tr>
<tr>
<td>Viability assay</td>
<td>Dye exclusion (light microscopy), fluorescence microscopy, flow cytometry</td>
<td>Dye exclusion and fluorescence microscopy provide nucleated cell viability data. Only flow cytometric analysis is capable of providing a viability result for HSCs (CD34+ cells).</td>
</tr>
<tr>
<td>Clonogenic assay</td>
<td>CFU (most common in clinical lab), LTC-IC</td>
<td>Only truly functional QC test for HSCs. Requires 14–16 day incubation at 37°C in 5% CO2. May be particularly useful for HSCs stored for several months to years (i.e. UCB).</td>
</tr>
<tr>
<td>CD34+ cell enumeration</td>
<td>Flow cytometry (single or dual platform)</td>
<td>A standard but not a functional assay for HSC graft potency. Gram stain and endotoxin testing may be useful if result of culture not available and microbial testing result needed prior to release (as may be the case with a more-than-minimally manipulated product). Results of antibiotic sensitivities and notification of patient physician imperative when positive test result.</td>
</tr>
<tr>
<td>Sterility Testing</td>
<td>Aerobic/anaerobic culture</td>
<td></td>
</tr>
</tbody>
</table>

Table 24.2. Quality control testing of HSC. Modified from McKenna DH. Hematopoietic stem cell processing and storage. In Murphy MF, Pamphilon D (Eds): Practical Transfusion Medicine, 2nd edn. Blackwell Publishing 2005 Table 4.

REFERENCES


**SUGGESTED FURTHER READING**


Intensive chemo/radiotherapy followed by hematopoietic stem cell transplantation is used in the treatment of certain hematological and oncological diseases. Three different sources of hematopoietic progenitor cells (HPC) are available: bone marrow (BM), peripheral blood progenitor cells (PBPC), and umbilical cord blood (UCB). HPC can be obtained from the patient and reinfused at a later date (autologous) or from another person who is a histocompatible match and is related or unrelated to the patient (allogeneic).

In addition to the collection, processing and storage of HPC, their infusion is an important step during the transplantation procedure. Failing to receive a transplant of viable, functional hematopoietic cells after intensive chemo/radiotherapy can be fatal. The responsibility of the infusing team is to carefully thaw the HPC, if frozen, and infuse them as soon as possible in a safe method to reduce the risk of acute side effects without harming the viability of the stem cells at the same time. This chapter addresses these issues surrounding the infusion of bone marrow, peripheral blood progenitor cells, and umbilical cord blood.

**HPC CRYOPRESERVATION**

In the setting of autologous BM or PBPC transplantation, the time interval between collection of HPC and transplantation can be from a few days to several weeks. To allow these prolonged storage times, a cryopreservative is added and the HPC product is stored at −196°C in the vapor phase of liquid nitrogen. The most widely used cryoprotectant is dimethyl sulfoxide (DMSO). Occasionally, hydroxy ethyl starch (HES) is used in combination with DMSO.

Granulocytes are disrupted during the freezing process. Red cells also lyze during the freezing and thawing process. Thus, the thawed HPC contains granulocyte debris (membrane fragments and enzymes), red cell stroma and free hemoglobin. These contaminants and the presence of the cryoprotectant necessitate special precautions during and after the infusion of the HPC product into the patient.

DMSO is eliminated by urinary excretion after conversion to dimethylsulfon (DMSO₂) and dimethylsulfide (DMSH₂). Renal excretion accounts for 44 percent of the administered DMSO dose. The plasma half-life (t½) of DMSO is about 20 hours. In contrast, the t½ of DMSO₂, a renal excreted metabolite, is 72 hours. A small portion of DMSO is reduced to DMSH₂, which is exhaled through the lungs for about 24 hours after HPC infusion accounting for the characteristic odor of the patient’s breath and permeating the patient’s room.

Several side effects have been described during cutaneous application and intravenous infusion of DMSO (Table 25.1). They include intravascular hemolysis, hyperosmolality, and increased serum transaminase levels. The LD₅₀ values (amount of DMSO required to kill 50 percent of test animals) reported for IV infusion of DMSO are 2.5 g/kg for dogs and 11 g/kg for monkeys.
HES has fewer side effects. Adverse reactions too are rare but include anaphylactoid reactions and prolongation of coagulation tests.\textsuperscript{10-12}

**SIDE EFFECTS OF INFUSING CRYOPRESERVED BM/PBPC**

Infusion of cryopreserved HPC is associated with a variety of symptoms. Side effects are usually mild to moderate; but very rarely life-threatening cardiac, neurologic, pulmonary, anaphylactic and renal events have been reported.\textsuperscript{6,13-17} In the largest study published to date, severe reactions occurred in only 0.4 percent of 1,410 patients.\textsuperscript{18} The most frequent symptoms are nausea, vomiting, hypertension and headache (Table 25.2) and these are most likely related to the DMSO. Abdominal cramps, diarrhea, flushing, and chest tightness have also been reported (Table 25.3). It is very common for patients to notice red urine within hours of the infusion depending on the content of lyzed red cells in the transfused product.\textsuperscript{19-22} It is also very common to notice a taste or odor described by some patients as as an aroma somewhat like garlic or sweet cream corn. This is presumably caused by the expired DMSH\textsubscript{2}. Patients receiving fresh BM have shown to experience fewer side effects than those receiving cryopreserved autologous BM.\textsuperscript{23,24} This is in agreement with observations that patients receiving cryopreserved autologous PBPC containing a higher dose of DMSO have shown more side effects than patients receiving a smaller volume of marrow with less DMSO.\textsuperscript{20,25} This suggests that DMSO is mainly responsible for those symptoms. Nevertheless, other factors might also contribute to the occurrence of the observed side effects. A higher incidence of cardiac toxicity was found in patients with prior exposure to cyclophosphamide,\textsuperscript{26} a high adriamycin dose prior to BMT,\textsuperscript{27} and a high red cell content of the HPC product.\textsuperscript{21} One study found a lower degree of toxicity in recipients of grafts of cells that were density-gradient separated and containing lower volume, including less DMSO and less cell debris as compared to products that were buffy-coat separated.\textsuperscript{25} The majority of cardiac side effects is self-limited and not associated with symptoms or mortality. They occur within the first seven hours after the infusion. However, some reports describe patients requiring specific treatment for bradycardia or even cardiac arrest immediately after the infusion.\textsuperscript{13,14,20,28}

Removing the liquid supernatant by washing of the cells prior to infusion might be an option for preventing life-threatening side effects occurring despite of antihistamine or antiemetic premedication. This must be weighed against the risks of cell loss. Reported recovery rates range from 73.9 + /– 6.4 percent of CFU-GM for BM to 93.9 + /– 7 percent for PBPC. In one study, an additional loss of 20 to 25 percent of the CFU-GM was observed from storage of the washed product for 4 hours at room temperature.\textsuperscript{29}

**Patient Premedication**

Patients receiving cryopreserved HPC are commonly premedicated (Table 25.3). Antihistamines and corticosteroids are most commonly used to prevent reactions due to DMSO, which could interrupt the infusion. Patients might also benefit from the administration of antiemetics or antipyretics to reduce nausea and febrile reactions.

Free hemoglobin and hemoglobinuria is potentially toxic to the kidney but the amount is usually small. Depending on the amount of red cells in the product, this risk might be important particularly in patients with pre-existing compromised renal function. Nevertheless, all patients should be well hydrated before and after the transplantation to prevent kidney damage, which if it occurred or was present would additionally interfere with the excretion of DMSO.

Depending on the number of autologous collections needed to obtain the desired dose of HPC, some autologous HPC have a large volume and as with other blood transfusions, the infusion of HPC can

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**Table 25.2:** Comparison of adverse reactions observed during infusion of the various types of stem cells: for cryopreserved bone marrow (BM)/peripheral blood progenitor cells (PBPC),\textsuperscript{18,20-23,25,27,28,68} noncryopreserved BM,\textsuperscript{23} and umbilical cord blood (UCB)\textsuperscript{55}

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Cryopreserved BM/PBPC</th>
<th>Noncryopreserved BM</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nause</td>
<td>38–77%</td>
<td>14%</td>
<td>10%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>24–64 %</td>
<td>8.5%</td>
<td>5%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5–41%</td>
<td>-</td>
<td>24%</td>
</tr>
<tr>
<td>Hypotension</td>
<td>22%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>0–65%</td>
<td>-</td>
<td>2%</td>
</tr>
<tr>
<td>Chills</td>
<td>31–65%</td>
<td>1.4%</td>
<td>-</td>
</tr>
<tr>
<td>Fever</td>
<td>2–67%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) indicates not present or not assessed
Table 25.3: Infusion of hematopoietic progenitor cells

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Type</th>
<th>Premeds</th>
<th>Cryoprotectant</th>
<th>DMSO</th>
<th>Speed</th>
<th>Side effects</th>
<th>Patient monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alessandrino 99</td>
<td>51</td>
<td>auto-BM</td>
<td>Methylpred, 10% DMSO</td>
<td>Split if more than 24 ml</td>
<td>10 ml/min</td>
<td>19% non-card., 21% hypertension, 27.5% bradycardia, 10% chest discomfort</td>
<td>BP, RRq 5 min during, q 30 min × 4 hr, ECG pre + post infusion</td>
<td>BP, RRq 5 min during, q 30 min × 4 hr, ECG pre + post-infusion</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>auto-PBPC</td>
<td>Methylpred, 10% DMSO</td>
<td>Split if more than 24 ml</td>
<td>10 ml/min</td>
<td>8% non-cardio., 36% hypertension, 2.7% bradycardia, 1.3% chest discomfort</td>
<td></td>
<td>P/BP 15 min during, q 30 min × 2 hr, q1h × 4 hr</td>
</tr>
<tr>
<td>Davis 90</td>
<td>82</td>
<td>auto-BM</td>
<td>Mannitol, Hydrocortisone, Diphenhydramin, 10% DMSO</td>
<td>10–20 ml/min</td>
<td></td>
<td>Buffy coat: 38% nausea/vomiting 26% flushing, 20% abd.cramping, 12% chest tightness, 8% diarrhea, Density gradient: 8% nausea, 2% headache ~50% nausea/vomiting, 0.4% severe reactions (1 anaphylactic, 2 neurologic, 2 leukostasis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graves 98</td>
<td>1410</td>
<td>auto-BM</td>
<td>10% DMSO</td>
<td>&lt;1 g/kg</td>
<td></td>
<td>38% P/BP 15 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>/PBPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoyt 2000</td>
<td>179</td>
<td>auto-BM</td>
<td>10% DMSO</td>
<td>16–92 ml</td>
<td></td>
<td>8% non-cardio., P/RRq 5 min during, q 30 min × 4 hr, ECG pre + post infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>/PBPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kessinger 90</td>
<td>100</td>
<td>auto PBPC</td>
<td>10% DMSO or 5% DMSO + 5% HES</td>
<td>within 2–4 hr</td>
<td></td>
<td>92% Hb-uria, 77% nausea, 65% chills, 67% fever, 64% vomiting, 61% pulse rate increase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keung 94</td>
<td>17</td>
<td>auto-BM</td>
<td>Mannitol, Diphenhydramin, Hydrocortison, 5% DMSO + 6% HES</td>
<td>0.65 - 1.9 ml/kg</td>
<td>20–50 ml/min</td>
<td>82% cardiac arrhythmia, 65% sinus bradycardia, 24% 2.gr block, 41% hypertension</td>
<td>cardiac monitoring pre-6 hr after infusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>/PBPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lopez-Jimenez 94</td>
<td>29</td>
<td>auto-BM</td>
<td>3x.Diphenhydramin, 10% DMSO</td>
<td>45 ml (35–90) (incl 20 min. breaks)</td>
<td>5.5 ml/min</td>
<td>41% non-cardiovasc.tox., no bradycardia or heart block</td>
<td>P/RRq5 min during, q30 min × 2 hr, q6h × 24 hr, ECG pre- and next day</td>
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<td></td>
<td>15</td>
<td>allo-BM</td>
<td>none</td>
<td>5.5 ml/min (incl 20 min. breaks)</td>
<td>6% non-cardiovasc., no bradycardia or heart block</td>
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<tr>
<td>Martino 96</td>
<td>22</td>
<td>auto-BM</td>
<td>10% DMSO</td>
<td>0.2–0.7 ml/kg</td>
<td>1 ml/kg</td>
<td>31.8% asymptomatic sinus bradycardia 1/22 necessitates atropin</td>
<td></td>
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<td></td>
<td></td>
<td>/PBPC</td>
<td></td>
<td></td>
<td>q12 hr</td>
<td></td>
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</tr>
<tr>
<td>Okamoto 93</td>
<td>54</td>
<td>auto-PBPC</td>
<td>Hydroxyzine, 10% DMSO</td>
<td>mean of 0.91 g/kg</td>
<td></td>
<td>74% Hb-uria, 70% headache, 69% nausea, 46% vomiting, 15% schock</td>
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<tr>
<td></td>
<td></td>
<td>Hydrocortisone</td>
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<tr>
<td>Smith 87</td>
<td>33</td>
<td>auto-BMT</td>
<td>10% DMSO</td>
<td>64–96 ml (3 cases)</td>
<td></td>
<td>3/33 renal toxicity</td>
<td></td>
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</tbody>
</table>
cause volume overload. Hypertension is observed in 21 to 41 percent of the patients. If the volume to be infused is sufficient to create a risk of volume overload, prophylactic diuretics can be administered. In addition, the infusion can be divided, thawed separately and infused during two separate times during the day of infusion. This also helps tolerate the DMSO load better.

Currently no studies are available to compare different premedication regimens. A comparison is difficult because many variables (DMSO dose, infusion speed, red cell content, etc.) differ between the transfusion centers.

### Preparing for the Transplant and Thawing of the HPCs

When the transplant is to be done, the product needs to be brought from the laboratory to the patient. It is most likely that the frozen product needs to be kept outside the liquid nitrogen storage tank for a certain period of time. Therefore, appropriate temporary storage is important. To avoid the risks associated with the use of liquid nitrogen during the transport from the laboratory to the patient, the bags can be transported at −79°C on dry ice (solid carbon dioxide). Nevertheless, delays should be avoided. One study found a considerable loss of CFU-GM after storing the cells for 24 hours at −79°C.30 A preferred temporary storage is the use of liquid nitrogen and commercially available dry shippers are available. These, though expensive, avoid the risks of spillage associated with the temporary use of small containers of liquid nitrogen.

With cooling at slow rates used to process HPC, intracellular ice nucleation is limited and mechanical disruption of the cell during warming is less likely compared to rapid rates of cooling. One study using glycerol as cryoprotectant found no difference in CFU-recovery for cells cooled at 1.7°C/min and warmed at either 1.8°C or 9 to 10°C/min.31 However, one report using DMSO describes a lower viability of CD34+ cells after controlled rate freezing and thawing at 4°C compared to thawing at 37°C.32

Usually, the bags are thawed in close proximity to the patient’s room using a 37°C water bath to shorten the time that the thawed HPC are exposed to DMSO. Rarely, freezing bags might break causing leakage. Therefore, the storage bag should be placed into a second bag during thawing.

Douay et al33 reported a substantial loss of granulocyte-macrophage precursor recovery when the cells were kept in DMSO at 4°C for a short time period (loss of 51% after 15 min, loss of 85% after 120 min). Other authors could not confirm these findings. They found no toxicity after incubation for up to 2 hours at 4°C or 1 hour at 37°C.34,35

Clumping of the product may also be a significant problem. This phenomenon has been explained by

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Type</th>
<th>Premeds</th>
<th>Cryoprotectant</th>
<th>DMSO Speed</th>
<th>Side effects</th>
<th>Patient monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroncek 91</td>
<td>134</td>
<td>auto-BM</td>
<td>10% DMSO</td>
<td>1.4–2.4 mL/kg</td>
<td>2–5 min/?</td>
<td>44.8% nausea, 23.9% vomiting, 31.3% chills, 17.9% fever</td>
<td>Vital signs at least 30 min after infusion</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>allo-BM</td>
<td>None</td>
<td>None</td>
<td></td>
<td>48% bradycardia start 0.5–7 hr after infusion, 9.7% high grade heart block</td>
<td>Vital signs at least 30 min after infusion</td>
</tr>
<tr>
<td>Styler 92</td>
<td>42</td>
<td>auto-BM</td>
<td>No</td>
<td>?</td>
<td>20 ml/min</td>
<td>45% nausea, 22% flushing, 12% fever, 9% chest tightness, 9% abd.cranps, 4.5% vertigo, 40% Hb-uria, 22% hypotension, 4.5% bradycardia</td>
<td>P/RR 15 min during, q30 min × 2 hr</td>
</tr>
<tr>
<td>Zambelli 98</td>
<td>22</td>
<td>auto-PBPC</td>
<td>Mannitol, Hydrocortisone, Chlorophenamine</td>
<td>10% DMSO</td>
<td>14–70 mL (mean 31.2 mL)</td>
<td>45% nausea, 22% flushing, 12% fever, 9% chest tightness, 9% abd.cranps, 4.5% vertigo, 40% Hb-uria, 22% hypotension, 4.5% bradycardia</td>
<td>Cont. ECG during-12 hr after, HR+BP q 5 min during, q30 min × 2 hr</td>
</tr>
</tbody>
</table>

BM: Bone marrow; PBPC: Peripheral blood progenitor cells; auto: autologous; allo: allogeneous; DMSO: dimethyl sulfoxide; HES: hydroxyethyl starch
breakdown of mature granulocytes and release of DNA.\textsuperscript{36} Based on these reports, it is a common practice, to start the infusion within a few minutes after thawing to minimize the exposure of non-frozen cells to DMSO.

**INFUSION OF HEMATOPOIETIC STEM CELLS**

Cryopreserved HPC should be administered through a central venous line. A 10 percent DMSO solution has a high molality (1.42 M) that can cause pain when infused through a peripheral line. Central lines in a patient undergoing autologous PBPC transplantation must meet various criteria. For the PBPC collection, a double lumen, large bore, stiff catheter is needed to allow a high flow rate. In some patients with good peripheral veins, two large bore peripheral lines can be sufficient for the PBPC collection. Later, in the course of therapy, a multilumen, long-term catheter is needed for chemotherapy, transplantation and intensive supportive care. Therefore, the transplant procedure traditionally involves the insertion of two different central venous lines. Lazarus et al\textsuperscript{37} report their experience with dual-lumen large-bore silastic multi-purpose ("hybrid") catheters, which they used as a single device for PBPC collection and supportive care during and after the transplantation. In 32/112 (29\%) catheter dysfunction occurred, 7/112 (6.3\%) catheters were removed because of infection or mechanical problems. Other authors report a similar complication rate in patients using a single double lumen catheter for apheresis and PBPC transplantation.\textsuperscript{38} In 7.1\% the catheter was removed because of thrombosis or infection, in 32 percent dysfunction occurred. Both authors concluded that the use of one catheter for stem cell collection and transplantation should be considered. The rate of catheter dysfunction in patients using the central venous line for BMT only varies between 7 and 52 percent.\textsuperscript{39-42} A recent report describes the use of peripherally inserted central lines (PICC lines) for autologous PBPC transplantation in patients with hematological malignancies. The incidence of phlebitis was 7.6\% (5/65) after a median of 8.9 days.\textsuperscript{43}

The product can be administered after transfer from the storage bag into a syringe or preferably, directly from the bag. The advantage of using the direct infusion from the bag is avoiding the risk of contamination and cell loss during transfer from bag to syringe. After the infusion, bag and line can be flushed with normal saline to minimize cell loss. The line can also be flushed during the infusion to increase the infusion rate; infusion pumps are normally not used to avoid cell damage. However, one study failed to demonstrate a significant difference between IV push, IV pump, and baseline thaw samples of PBPC.\textsuperscript{44}

There is agreement that the HPC product should never be irradiated or transfused through a leukoreduction filter. These procedures would damage or remove the HPC. Less clear is, whether a routine 170 μm blood filter should be used or not. Rowley\textsuperscript{45} recommends the use of filters to remove clumps, Trealeaven\textsuperscript{46} prefers to transfuse bone marrow without a filter, because the filter might be blocked by cellular elements and debris delaying the infusion and possibly trapping HPCs. No studies are available to compare both options.

In order to minimize the time of contact between the thawed HPCs and DMSO, there is an incentive to infuse the HPCs as rapidly as possible. The factors to consider in determining the rate of HPC infusion are: (i) volume of HPCs in relation to the patient’s blood volume, (ii) amount of DMSO in the HPC product, and (iii) temperature of the HPC product. The infusion rate of 10 percent DMSO cryopreserved products reported in the literature varies between 5 and 20 ml/min. In the study with the lowest incidence of bradycardia and heart blocks, the authors attribute this to a slower infusion rate.\textsuperscript{24} They infused bone marrow with 20-minute breaks between the aliquots, resulting in an infusion rate of 5.5 to 6 ml/min. One study used an infusion rate of 20 to 50 ml/min in 5 percent DMSO cryopreserved BM or PBPC in 17 patients.\textsuperscript{27} They report a relatively high incidence of cardiac arrhythmias and hypertension (82\% and 41\% respectively). These results suggest that acute volume expansion leading to reflex slowing of the heart rate, and vagal responses to the coldness of the freshly thawed product can be responsible for cardiac side effects independently from DMSO. Nevertheless, some patients in this study experienced cardiac side effects starting as long as 7 hours after the infusion, which cannot be explained by the cold temperature or volume of the infusion.

The dose of DMSO given during the infusion of thawed BM or PBPC varies. Some studies report doses between ~0.2 and 0.7 g/kg of the recipient, some
Infusion of Hematopoietic Stem Cells

report absolute doses between 15 and 92 ml DMSO, which may equal up to 1 ml per kg in a normal size adult. In one study, the product was split, if more than 240 ml (equivalent to 24 ml DMSO) were infused, other authors used an upper limit of 1 ml DMSO per kg of patient weight. To avoid exceeding this threshold, products were divided and infused at 12- to 24-hour intervals. Using this limit, they found that ~50 percent of the patients experienced nausea/vomiting, which is comparable to or slightly higher than in other studies, and only 0.4 percent of 1,410 patients experienced severe reactions.

Most commonly, HPCs are cryopreserved in 10 percent DMSO. However, some studies show satisfactory results after using 5 percent DMSO as cryoprotectant and storage for several months. Additional studies are needed before this procedure can be recommended for cryopreservation for longer time periods, which would reduce the amount of DMSO infused.

Patient Monitoring During and After the Infusion

Because of the above-described possible side effects, patients are monitored during and after the infusion. Usually, blood pressure and pulse are monitored closely during the infusion (every 5–15 min), followed by measurements every 30 to 60 minutes over the following 6 hours, sometimes up to 24 hours. An ECG is sometimes performed prior to and immediately after the infusion, sometimes on the following day or during the infusion (Table 25.3). Non-cardiogenic side effects usually occur during the infusion and they resolve after the infusion is stopped. However, some reports describe the need of atropine treatment to resolve bradycardia. Two patients were treated within 4 hours, one within 6 and one within 12 hours. Therefore, it seems reasonable to monitor the patients for several hours after the transplantation. More frequent vital signs may be required depending on reactions to the product infusion.

SIDE EFFECTS OF NON-CRYOPRESERVED PBPC/BM INFUSION

In allogeneic BM or PBPC transplantation, the collected HPC are not cryopreserved and are transfused shortly after the collection. They are usually kept at room temperature until they are infused. However, transplantation with allogeneic cryopreserved marrow has been described in some cases. The infusion of a non-cryopreserved product is generally better tolerated (Table 25.2).

Because of the importance of HLA matching in HPC transplantation, products may be transfused despite ABO incompatibility. In the case of major incompatibility, the immediate risk is acute hemolysis of the transfused red cells. The risk is lower in recipients of PBPC, because of a lower red cell content in this product (hematocrit 2–5 percent; volume 75–100 ml) compared to bone marrow components (hematocrit 25–35%; volume 300–400 ml). The amount of red cells present in the later can be reduced by sedimentation techniques.

Patients receiving a minor ABO-incompatible graft may experience immediate hemolysis from infusion of plasma that is incompatible with the recipient’s red cells. This risk is generally low, but varies with volume of infused plasma, isoagglutinin titer of the graft, and body size of the recipient. Depletion of plasma can be performed to lower the amount of isoagglutinins infused. Delayed hemolysis caused by transfused viable lymphocytes producing isoagglutinins seems to be of greater clinical importance in those patients. Several cases of severe hemolysis have been reported.

Nevertheless, a retrospective study looking at 158 recipients of ABO-incompatible BM (90) or PBPC (68) did not find evidence of hemolysis in the recipients within 21 days after transplantation. Major ABO-incompatible BM components were red cell depleted resulting in a RBC content of <15 ml, if the recipient’s isoagglutinin titer exceeded 1 : 16. Minor ABO-incompatible BM components were plasma depleted, if the donor isoagglutinin titer exceeded 1 : 128. PBPC were not manipulated.

A recent case report describes a transfusion-related acute lung injury (TRALI) after the infusion of allogeneic bone marrow.

PREMEDICATION, INFUSION OF NON-CRYOPRESERVED STEM CELLS AND PATIENT MONITORING

If fresh stem cells with no cryopreservative are infused, the patient should be premedicated if there is a history of blood transfusion reactions. In case of a
major ABO incompatibility between graft and recipient, the patient should always receive antipyretics and possibly antihistamines and should be well hydrated to prevent kidney damage should there be a large amount of hemolysis. The product can be infused directly from the bag. Fresh products do not contain a cryoprotectant. Therefore, the amount of DMSO contained does not limit the infusion rate or volume to be given. Nevertheless, the amount of plasma and red cells contained in a stem cell product should be considered in minor and major ABO-incompatible transplantations.

Although adverse reactions are not as frequent during the infusion of fresh products as in thawed cryopreserved HPC, the patient’s vital signs should be monitored as for any blood transfusion. Reported studies do not describe different protocols for fresh and thawed products.

**INFUSION OF UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS**

Adverse reactions associated with UCB infusion are less frequent and appear to be less severe than those reported for cryopreserved bone marrow or PBPC (Table 25.2). Cord blood is cryopreserved in 10 percent DMSO, but is usually washed after thawing to remove DMSO and free hemoglobin. The volume of the thawed cord blood unit tends to be small (60–120 ml). Therefore, a lower rate of side effects would be anticipated as compared to cryopreserved BM or PBPC. However, since many UCB recipients are small, the volume of the product and the amount of DMSO being infused must be related to the patient’s size. Regarding the infusion of UCB, the common recommendations for thawed and ABO-incompatible products apply. Because of the small volume, flushing of the bag and the infusion line should be considered after the infusion to minimize cell loss.

**Patient Identification and Inspection of the Product**

For all three types of HPC, it is critical to have special precautions in place to prevent clerical errors. There should be a written order from the physician to administer the HPCs. The product should be double checked with a second nurse/physician verifying that the identification number of the bags, the product hang tag, the patient and the physicians order are in accordance. At the same time, a visual inspection for product container integrity, product color and turbidity should be performed.

**CLINICAL MANAGEMENT OF MICROBIALLY CONTAMINATED HPC PRODUCTS**

Bacterial contamination of bone marrow or PBPC can occur during collection, *ex vivo* manipulation, storage, or the thawing and infusion process. The reported microbial contamination rates are between 4.4 and 17 percent for bone marrow, and 0.2 and 5.2 percent for PBPC products. The most common organisms identified are coagulase-negative *Staphylococcus* species and other skin flora. Gram negative organisms and fungi are less common. AABB standards state that nonconforming products should be administered only with informed consent of the recipient and approval by the physician. Some investigators have discarded contaminated collections. Several other reports show low rates of bacteremia without any serious sequelae in recipients of contaminated products, and there have not been any reported fatalities. However, often, cultures were not isolated in postcollection and post-thaw specimens and were considered insignificant. Some investigators also recommend organism-specific antibiotic coverage when infusing known contaminated products.

**REPORTING AND INVESTIGATION OF ADVERSE EVENTS RELATED TO THE INFUSION OF HPC**

Similar to transfusion of other blood components, the investigation and reporting of adverse events are important. AABB standards and FACT (Foundation of Accreditation of Cellular Therapy) state that the HPC and cellular product service shall establish a process for the detection, reporting, evaluation, and documenting of adverse reactions. Information about the transfusion of HPCs is an important part of the quality control program to provide the safest possible product for patients. All laboratories will have some sort of information sheet to obtain the patient care information that occurs during and after HPC infusion. This data is summarized and reviewed periodically as a part of the patient care quality program.
Severe adverse events should be reported to the laboratory immediately. Each laboratory will develop its own definition of severe adverse events, but this might include hypotension, temperature elevation greater than two degrees, cardiac arrhythmias, severe dyspnea or hemoglobinuria. Summarizing and monitoring this information and discussing it among laboratory and transplant staff makes it possible to use transfusion techniques that are as safe and comfortable as possible for the patient.

CONCLUSION

Although an HPC infusion can be seen as a special blood transfusion, it differs in several points. Thawed products contain DMSO, they are cold when infused, they have a high osmolality and they contain cell debris and some free hemoglobin. ABO-incompatible products are sometimes needed and infused because of the importance of HLA matching. Adding the cryoprotectant, alterations resulting from freezing and thawing, and transfusion across the ABO barrier require special precautions before, during and after the infusion.

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Bone marrow transplantation (BMT) today offers the possibility of cure for many acquired and genetic disorders of the hemopoietic stem cell. Stem cells for transplantation can be obtained from the bone marrow, the peripheral blood or from the placenta (umbilical cord blood). The physician who undertakes stem cell transplantation has to have a thorough understanding of blood banking. Transfusion of blood components is an essential part of the transplant procedure, especially during the period of aplasia, after chemotherapy or radiation has ablated the bone marrow and engraftment has not yet taken place. Collection and processing of the stem cells is an integral part of the transplant procedure and the transplantor must understand the principles involved.

This section has been written mainly for the hematologist working in the developing world who is planning to set up a transplant facility. In the developing world, the team leader has to be familiar with all aspects of the infrastructure needed for transplantation, and an attempt is made in this section to provide practical information on blood transfusion requirements of the patient undergoing stem cell transplantation.

Pre-Transplant Work up of the Patient

**History**

Detailed history of previous transfusion including reactions, use of leukodepletion and whether transfused from first degree relatives. This history is particularly relevant for the patient with thalassemia who is to have BMT.

**Laboratory Tests**

1. CBC, blood film review by consultant, reticulocyte count
2. Blood Group
3. Red cell phenotype if not recently transfused and if ABO same as donor
4. Anti-A/anti-B titers in ABO mismatched transplants
5. Recipient-donor cross match in ABO matched transplants
6. **Viral Screen:** HbsAg and anti-HBs, HIV 1 and 2, HCV antibody.

**Donor**

**History**

A detailed history is required, in particular transfusion history, medications, risk factors for HIV and other viruses and in the case of adult females, the date of the last menstrual period and pregnancy history.

**Physical Examination**

Detailed physical examination and check for availability of good veins if peripheral blood stem cell collection is planned.
Laboratory Tests
1. CBC and blood film reviewed by the consultant
2. Blood Group
3. Red cell phenotype if same ABO group as patient
4. Anti-A/Anti-B titers if ABO mismatched transplant
5. Recipient-donor cross match if ABO matched transplant.

Autologous Blood
All adult donors should have 1 to 3 units of autologous blood stored prior to the marrow harvest depending upon the amount of marrow to be harvested. In the case of donors below the age of 5 to 6 years where it may not be possible to collect autologous blood, homologous blood should be kept cross-matched and irradiated in the blood bank for the harvest but used only if absolutely necessary.

Providing Blood and Platelet Donors for Transfusing Following the BMT
A minimum of 2 healthy donors should be screened and available for platelet apheresis. This is necessary in the developing world since volunteer donors may not be available for apheresis in an emergency when the blood bank is not able to provide the components necessary for the transplant patient.

Venous Access
All transplant patients will require the insertion of a Hickman catheter for optimum venous access during the transplant period. A dual lumen Hickman catheter is inserted by the surgeon or by the radiologist percutaneously.

Coagulation profile should be checked prior to surgery.

Platelet Count
The platelet count should be at least >50 × 10⁹/L for the surgery. Appropriate platelet transfusions should be instituted if necessary and platelet refractoriness determined well in advance of the surgery. In patients who do not get optimum increments to platelet transfusions, 6 to 8 units of platelets should be transfused in the OR just before the procedure and further aggressive platelet support given postoperatively if required.

Coagulation Studies
If prolonged, should be corrected prior to surgery. In chronically ill patients, vitamin K deficiency may be a possibility and the administration of 10 mg of vitamin KI IV may correct the coagulation abnormality. If not 20 ml/kg of fresh frozen plasma (FFP) may be transfused before surgery and the coagulation parameters checked before sending the patient to the OR.

TRANSFUSION SUPPORT FOR THE BONE MARROW HARVEST
The volume of blood required for the bone marrow harvest can be calculated based on the weight of the donor, the recipient and the average total nucleated cell count (TNC) of the harvested bone marrow. In general, in an adult, the TNC in the bone marrow harvest is around 15 × 10⁸/L; while in a child, it is usually 20 × 10⁸/L. If G-CSF is administered to the donor prior to the harvest, then the counts are much higher.

Given below are examples of the calculation of blood required for a donor harvest:
1. Recipient weight 80 kg: TNC required= 3 × 10⁸/kg × 80 kg = 240 × 10⁸/kg or 24 × 10⁹ cells = 1000 ml of bone marrow if the count is 24 × 10⁹/L or 2 liters if the count is 12 × 10⁹/L
   Donor weight 60 kg with Hb of 14 g/dl: 1000 ml of bone marrow can be harvested with crystalloid replacement; but if the donor is older, then one would keep two units of autologous blood available in case a 2-liter harvest has to be done. Bone marrow tends to be less cellular with advancing age and, therefore, the TNC in the harvest is lower.
2. Recipient weight 30 kg: TNC required = 3 × 10⁸/kg × 30 kg = 90 × 10⁸/kg or 9 × 10⁹ cells = 500 ml of bone marrow if the count is 18 × 10⁹/L.
   Donor weight 10 kg with Hb of 10 g/dl: No more than 20 percent of the circulating volume of 1000 ml of harvest may be permitted without replacement: therefore, at least 250 ml of whole blood/packed cells need to be available for the bone marrow harvest.

Our policy is to irradiate and leukodeplete all cellular blood products that are transfused to the donor during the harvest in order to reduce the risk of alloimmunization. The leukodepletion is done during the harvest with a filter (Pall RC100).
ABO MISMATCHED TRANSPLANTS

In allogeneic BMT, ABO incompatibility between donor and recipient does not affect the long-term success of BMT, or the incidence of graft failure or GVHD. Hemopoietic stem cells do not carry ABO antigens and, therefore, transplantation of ABO mismatched donor recipient pairs is possible unlike solid organ transplants where endothelial cells carry ABO antigens which will react with preformed ABO antibodies in the recipient. However, in hemopoietic stem cell transplants careful transfusion practice is essential to prevent hemolytic transfusion reactions. When transfusing a marrow transplant recipient, it is essential that both the clinician and the blood bank know the donor’s blood group.

**Major ABO Mismatch:** Recipient has antibodies to donor ABO antigens. For example, O recipient with A donor.

**Minor ABO Mismatch:** Donor has antibodies to recipient’s ABO antigens. For example, B recipient with a O donor.

**Major ABO Mismatch**

To prevent a hemolytic transfusion reaction during the marrow infusion, the **RECIPIENT’S** antibody should be removed by plasma exchange and/or by transfusion of donor ABO group secretor plasma. **OR** the **DONOR’S** red cells in the harvested marrow are removed by sedimentation prior to the infusion.

a. Procedure for removal of recipient anti-A/B when there is an ABO major mismatch.
   - “Safe” levels of antibody are titers of < 1 : 8 (IgG) or < 1 : 16 (IgM). If levels are higher than this, the following procedure is used:
     1. Donor group specific secretor plasma 1 to 2 units are given daily a few days prior to the transplant and titers rechecked.
     2. If titers are still very high, then plasma exchange is performed pre-transplant with the aim of removing 3 to 5 liters.
   - However, if the bone marrow harvest is adequately red cell depleted, it may not be necessary to reduce the ABO antibody levels in the recipient. In fact, most transplant centers today would only deplete the harvested stem cell product of red cells and not do anything about the recipient’s ABO antibody.

b. Procedure for removal of donor red cells **in vitro** when there is an ABO major mismatch.
   - There are many techniques available for red cell depletion of the bone marrow. Refer to Appendix C.
     - Gravity sedimentation using hydroxyethyl starch.
     - Gravity sedimentation using dextran
     - Processing of the marrow on an apheresis machine, e.g. Cobe Spectra using the White Cell Protocol.
   - Institutional guidelines for maximum red blood cell content of ABO incompatible stem cell products vary considerably as given below:
     - 20 ml (adults), 30 ml (adults), 0.15 ml/kg, 0.25 ml/kg (with pediatrics, no less than 5 ml due to recovery loss), 0.30 ml/kg, 1 ml/kg (pediatrics), “by directive of clinician” (pediatrics), threshold may be exceeded in the adult setting (>20 ml) depending on isohemagglutinin titers (recipient) against the product red cell type. If <1 : 16, then more RBCs are infused.

c. Infusion of ABO major mismatched marrow
   - A good IV fluid input is essential. Give small doses of frusemide if necessary to establish a diuresis. Some centers would use mannitol to ensure diuresis and prevent ABO mismatch-related acute tubular necrosis.

**Minor ABO Mismatch**

If donor anti-A/B titers are high, i.e. > 1 : 16 (IgG) and >1 : 32 (IgM), the marrow should be plasma reduced (Appendix D).
   - Maximum plasma content (minor incompatibility).
     - No depletion if plasma volume is 250 to 275 ml, plasma volume depletion is dependent upon donor antibody titers. **Adults:** greater than 1 : 256, plasma deplete; **Pediatrics:** plasma deplete mainly for volume reduction.

**CHOICE OF ABO GROUP OF BLOOD PRODUCTS IN ABO AND RHESUS (D) MISMATCHED BMT**

Obviously, if donor and recipient are of the same group, then group specific blood products are preferred. Table 26.1 gives the choice of red cells to be used in an ABO mismatched transplant particularly during the peri-transplant period while the group of the recipient is changing to the group of the donor.
Table 26.1: Group of blood products in ABO and RH (D) mismatched BMT recipients

<table>
<thead>
<tr>
<th>Group donor</th>
<th>Group recipient</th>
<th>Packed red cells</th>
<th>Platelets and granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major ABO Mismatch</td>
<td></td>
<td></td>
<td></td>
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* significant risk of hemolysis

**STEM CELL COLLECTION**

Stem cells can be harvested from the bone marrow, the peripheral blood or the umbilical cord. In an allogeneic stem cell transplant the exact number of long-term repopulating stem cells is difficult to determine but most centers would attempt to transfuse:

- $3 \times 10^8$ total nucleated cell/kg recipient weight
- $3 \times 10^6$ CD34+ cells/kg recipient weight
- $3 \times 10^4$ CFU-GM/kg recipient weight.

**Bone Marrow**

About 1 to 2 percent of the total nucleated cells in the bone marrow harvest are CD34 positive. The technique for harvesting is described in Appendix B. In general, the bone marrow harvest has fewer T-cells when compared to a peripheral blood stem cell harvest and there is data that chronic graft-versus-host disease is lower. However, engraftment is slower and graft versus leukemia effects may be less with bone marrow as the source of stem cells. In our center, we would use bone marrow as the source of stem cells in the following situations:

- CML in chronic phase
- Minimally transfused aplastic anemia
- Transplants in patients with thalassemia

**Peripheral Blood Stem Cells**

Stem cells are present in the peripheral blood in very small numbers in normal individuals: this number is increased during recovery following intensive chemotherapy and after administration of growth factors like G-CSF. For an autologous transplant, stem cells may be collected during the recovery phase after chemotherapy and growth factors have been administered after measuring the number of CD34 cells in the peripheral blood. In the allogeneic setting stem cells are collected after administration of growth factor usually G-CSF 10 micrograms/day for 4 to 5 days and the collection is done on day 5. Enough blood is processed to obtain a mononuclear cell dose of $3 \times 10^8$ MNC/kg recipient weight. Large volume leukapheresis may be performed where the CD34 cells requirements are high as in a haploidentical transplant.

**Umbilical Cord Blood**

The umbilical cord is a rich source of stem cells and can be used for transplantation where an HLA-matched sibling donor is not available. The engraftment is slower and, therefore, the transfusion support required will be much greater in umbilical cord blood transplants.

Stem cell collection and processing are covered in detail in separate chapters of this book.

**STEM CELL INFUSION**

**Marrow Infusion**

1. The marrow is usually transfused soon after the harvest unless red cell or plasma depletion needs to be done.
2. If the marrow needs to be plasma reduced or red cell depleted, the appropriate protocol is followed prior to infusion (Appendices C and D).
3. Check vital signs and auscultate the chest prior to the infusion.
4. In general, no premedication is needed; but if the patient has been multiply transfused in the past and has had febrile transfusion reactions, premedication with an antihistamine like diphenhydramine may be given.

5. The marrow is infused through a regular blood transfusion (170 micron filter) set over a period of about 4 hours. Never use a leukocyte filter for marrow infusion. Infuse as slowly as possible for the first 15 minutes.

6. Watch for fluid overload—give a diuretic if necessary.

7. The BP and pulse will be monitored every 15 minutes during the infusion.

Complications of Marrow Infusion

1. Pyrexia, rash and rigors can occur and should be treated with meperidine (pethidine), antihistamines and or paracetamol. In children hyperpyrexia can precipitate febrile seizures. This should be managed aggressively with intravenous diazepam and tepid sponging.

2. Hemolytic transfusion reactions can occur in ABO mismatched transfusions. If it is suspected clinically, the infusion should be stopped, the patient hydrated and urine and plasma checked for hemoglobin.

3. Microemboli occasionally cause dyspnea and cyanosis. Oxygen should be available. Slow down the marrow infusion if dyspnea occurs.

4. Acute anaphylaxis is very rare but 1 ml of 1:10,000 adrenaline should be available for immediate administration.

More details of stem cell infusion are given in a separate chapter of this book.

BLOOD PRODUCT SUPPORT FOR THE RECIPIENT

Irradiation

All blood products given to BMT patients must be irradiated to prevent third party transfusion-related graft-versus-host disease. Each bag is irradiated to 2,500 cGy from day minus 6 until at least 2 years after BMT. Irradiated red blood cells release potassium relatively quickly such that by day five, the potassium concentration in the bag approaches that of unirradiated stored blood at expiry (Day 35). In general, therefore, red cell transfusion support should be anticipated and blood irradiated a day earlier or on the day of the transfusion.

Irradiation can be done using the Nordion Blood Irradiator which uses cesium as the source. The equipment is expensive. The Atomic Energy Commission of India produces a low cost blood irradiator where cobalt-60 is the source. Irradiators which use X-rays are also available and these equipment have less stringent regulatory requirements. Where a dedicated blood irradiator is not available, it is possible to use the patient irradiator for blood products though this is not ideal.

Prior to transfusion, the irradiation status of the unit to be transfused is checked by the transplant nurse and physician and documented on the appropriate flow sheet. This is to avoid the inadvertent transfusion of unirradiated blood products.

Indications for Blood Product Transfusion

Platelet Transfusions

Prophylactic platelet transfusions will be given if the platelet count is < 20,000/mm³. This usually means the transfusion of 4 to 6 units of random or single donor platelets. If the patient has major hemorrhages, the intensity of platelet support will increase. There is some debate as to whether the trigger for platelet transfusion postallogeneic stem cell transplant can be reduced to 10,000/cumm. One of the main problems in the recipient is the development of platelet refractoriness due to alloantibodies. In this situation, the blood bank can do one of the following:

- platelet cross match and provide compatible random donor platelets
- collect platelets from HLA-matched donors
- collect platelets from the stem cell donor who is HLA matched if the donor is an adult.

Red Cell Transfusions

In general, packed cells will be transfused to maintain the hemoglobin above 8 g/dl or if the patient is symptomatically anemic.

Fresh Frozen Plasma

If the patient is bleeding due to a coagulopathy, then FFP is transfused. FFP does not need irradiation.
Granulocyte Transfusions

If the patient is neutropenic and has uncontrolled sepsis despite adequate antimicrobial support, granulocyte transfusions may be given. There is no randomized controlled study which shows that granulocyte transfusions are beneficial for septic neutropenic patients. However, administration of growth factor (G-CSF) and dexamethasone now allows collection of larger numbers of granulocytes and these transfusions may tide over a crisis particularly in patients with aplastic anemia and fungal sepsis at the time that conditioning has begun and there is still a 2-week period to tide over before engraftment.

Bedside leukodepletion for red cell and platelet transfusion can be done using appropriate filters. Leukodepletion has the following benefits:

- Reduction of febrile transfusion reactions.
- Reduction of CMV transmission.
- Reduction of alloimmunization.

In the developing world, leukodepletion of all cellular blood products increases the cost considerably particularly when transfusion requirements are high.

Transfusion Support for Veno-Occlusive Disease of the Liver

Pathophysiology and Clinical Diagnostic Criteria

Hepatic veno-occlusive disease (VOD) refers to the clinical syndrome that results from obliteration of terminal hepatic venules and small sublobular veins. VOD is seen in approximately 20 percent of BMT recipients following high-dose chemotherapy/radiotherapy given as pre-transplant conditioning. The clinical symptoms usually appear between the first and third week of post-transplant and consist of:

1. Hyperbilirubinemia often with a disproportionately low enzyme elevation.
2. Tender or clinically enlarged liver.
3. Fluid retention in the form of ascites associated with a significant weight gain.

In patients with VOD, there is significant consumption of platelets and it is difficult to maintain platelet counts because transfusion does not produce the required increment in platelet count. Coagulation parameters are often deranged and fresh frozen plasma is required.

Hemorrhagic Cystitis

This is a complication related to acrolein, a product of cyclophosphamide metabolism. However, in late onset hemorrhagic cystitis, infection with BK and adenoviruses can contribute. Often, this complication is accompanied by sepsis and graft-versus-host disease and reduction in platelet counts. Platelet transfusions to maintain counts in the range of 60,000/cumm can reduce the bleeding. In severe cases, red cell transfusion requirements are high.

Transfusion and CMV Status of Recipient and Donor

In the developing world, most adults are CMV IgG positive indicating previous infection. Therefore, it is extremely difficult to get CMV negative blood components. This may not be important since most patients and their donors are also CMV IgG positive. In the Western world, 20 to 30 percent of the population has never been infected with CMV and, therefore, patients and donors may be CMV seronegative. In this situation, the blood transfusion service will provide seronegative blood components for the transplant patient.

POST-TRANSPLANT TRANSFUSION SUPPORT

In general, no blood or component transfusion is necessary once the patient returns home after a successful allogeneic stem cell transplant. However, there are some situations where transfusion support is required; and in these situations, the local physician needs to be aware:

- that the patient’s group has changed to that of the recipient in a blood group mismatched transplant and
- that the patient’s immune system takes about a year to fully recover and, therefore, it is recommended that only irradiated blood products should be transfused for a period of 2 years after transplant.

The following are the situations where transfusion may be necessary after allogeneic stem cell transplantation:

- Post-traumatic hemorrhage as for any normal person
- Graft failure with autologous recovery: this happens in thalassemia major transplants
• Post-transplant pure red cell aplasia usually in
ABO mismatched transplants where it may take
as long as a year for recipient antibodies against
the donor’s ABO antigens to reduce and donor
erthropoiesis to be established.
The same principles should guide transfusion
practice in this period as given in the section on
transfusion support of the patient.

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APPENDIX A

Bone Marrow Harvest Kit
The bone marrow harvest kit contains all the equip-
ment needed for the harvest. It should be checked a
week before and a day prior to the harvest to ensure
that all is in order.

Contents of Kit
1. Bone Marrow Needles
   a. Illinois Aspiration Needles × 4
   b. Adult Jamshidi Needles or × 4
   c. Pediatric Jamshidi Needles × 4
2. Collection Bag Stand
3. Marrow Collection Bags × 4
4. Disposable 10 ml Syringes × 30
5. #18 Disposable Needles × 2
6. Preservative Free Heparin 5000 U/ml × 6 amps
7. Bag Sealer and Sealing Clips
8. Leukocyte Filter (RC 100) × 1
9. ACD-A 500 ml × 4 bottles

Baxter produces a bone marrow collection system
which can be used for the harvest. However it is
possible to fabricate a simple collection system using a one liter transfer bag and fusing a blood transfusion set to the inlet port using a sterile docking device.

**APPENDIX B**

**Bone Marrow Harvest Procedure**

**Set up**

One transplant physician scrubs first and is handed the sterile equipment to be set up for the harvest:
1. A table at the foot end of the bed is draped by a sterile sheet and the collection bag stand assembled on it. The collection bag is hung up for use.
2. The following items are then made available on this table:
   - 50 cc disposable syringe × 1
   - 5 cc disposable syringe × 1
   - #18 insyte × 1
   - #20 disposable needle × 1
3. With the help of another physician, 100 cc of ACD-A is drawn into the 50 cc syringe using the #18 needle. Next, 5,000 U of preservative free heparin is added to the bag using the 5 cc syringe. The collection bag is now ready for 400 ml of marrow.

*Formula:* 5,000 U Preservative Free Heparin + 100 ml ACD-A every 400 ml of marrow
4. One liter normal saline is emptied into a stainless steel container on the sterile O.R. table. 500 ml of ACD-A is poured into another sterile basin. This will be used for rinsing the harvest syringes and needles.
5. The harvest syringes (10 cc disposable syringes × 20) and the harvest needles are then handed to the scrubbed up OR nurse to be rinsed with the ACD-A and kept ready for use.

**Positioning the Donor**

1. The patient is placed in the prone position once anesthetized.
2. Pillows are placed under the pelvis so as to elevate the posterior iliac crests.

**Heparinizing the Donor**

25 U/kg of preservative free heparin is injected IV by the anesthetist just prior to the harvest. This is in order to make sure that the marrow does not clot during the harvest.

**Harvest**

1. Three persons need to be scrubbed up for the procedure for an adult donor. Two for harvesting and one for collecting the marrow into the bag.
2. The harvest site is cleaned with betadine and draped.
3. The posterior superior iliac spine is identified on each side and a small stab incision made. The needle is advanced vertically 4 to 5 cm (depending upon the size of the donor) into the bone via the skin incision and marrow aspirated. Rotate the needle on its vertical axis 90 to 180 degrees if there is difficulty obtaining marrow initially. Remove the syringe and hand it over to the third physician who will empty it into the bag. Withdraw the needle 1 cm and aspirate as before. Aspirate only 2 to 4 ml of marrow from each level and from not more than 2 to 3 levels from each site. With the last aspirate, remove the needle and hand it over to the nurse who will rinse it and get it ready for re-use. Using the same incision, aspirate from different areas of the posterior iliac crest. If the sites are exhausted, make another incision a few centimeters away superolaterally along the iliac crest and aspirate. In the rare event that insufficient marrow is obtained posteriorly, the patient is turned over and aspirations done from the anterior iliac crest. If necessary, marrow aspiration may even be attempted from the sternum.
4. The assistant who empties the marrow into the bag calls out the volume of marrow in the syringe each time. This is noted down on the worksheet by a fourth person. Blood counts are done from the marrow harvest bag half-way through the harvest and after completion of the harvest.
5. The harvest is terminated once the appropriate volume of marrow has been obtained. The site is cleaned with surgical spirit. Gauze and pads are placed over the wounds and secured with adhesive.
6. The marrow bag is sealed and taken to the BMT unit. If red cell depletion or plasma reduction is required, it is taken to the laminar flow hood where processing is done.
APPENDIX C

Red Cell Depletion

Apheresis collection methods employed to minimize red cell contamination in the harvest.

- Gambro (Cobe) Spectra Version 4 (WBC-MNC procedure)
  - Product Hct: 2-5 percent
  - Product volume: 200-300 ml
- Gambro (Cobe) Spectra “AutoPBSC” procedure
  WBC-MNC procedure had a higher CD34 yield as opposed to the AutoPBSC procedure; however, platelet loss using AutoPBSC was much lower than with the WBC-MNC procedure.

APPENDIX D

Hydroxyethyl Starch Sedimentation for Red Cell Depletion

Principle

Hydroxyethyl starch coats the red blood cells causing agglutination. The red cells settle, leaving the nucleated cells in the supernatant. The agglutinated red cells are then drained off from the bag and discarded.

Technique

1. The procedure is done in the Bone Marrow Processing Laboratory in the laminar air flow hood.
2. Calculate the volume of marrow in the bag:
   Weight in gm/1.03 = volume in ml
3. Calculate the volume of HES needed:
   Vol of HES to add (ml) = vol of marrow (ml)/6
4. Add the appropriate volume of HES into the marrow bag using a disposable 50 cc syringe and mix well. If the marrow bag is too full to start with, transfer half the marrow to another bag and add HES to both in the calculated ratio.
5. Hang the marrow bag(s) with the ports oriented downwards. After the marrow has settled for 30 minutes, mark the side of the bag at the top of the buffy coat. Repeat every 10 minutes. When the sedimentation rate slows down (usually 45 min to 1 hr), drain off the red cells until about 1/4 inch of the buffy coat remains in the bag.
6. Clamp and seal the bag(s).

APPENDIX E

Plasma Reduction of Bone Marrow

Principle

In minor ABO mismatched bone marrow transplants, it may be necessary to remove the plasma from the donor marrow before infusion, if the donor anti-A/B titres are high (> 1 : 16 IgG and > 1 : 32 IgM). This is done by centrifugation and aspiration of the supernatant plasma.

Technique

1. Refrigerated centrifuge
   a. Temperature: 22°C.
   b. Speed: 4,000 rpm
   c. Time: 15 min
2. The bone marrow bag is placed in one of the centrifuge buckets. A bag with water is placed in the bucket diagonally opposite to that of the marrow bag to serve as a balance. The two buckets are then weighed and equalized using the plastic weights.
3. The buckets are put back in their respective slots and the centrifuge closed and turned on.
4. Once the centrifugation has been completed, the marrow bag is taken out and the outlet tubing connected to a transfer pack. Using the plasma expressor, the plasma is gently expressed into the transfer pack leaving behind about 1/4 inch of plasma in the marrow bag. The tubing is then sealed.
5. Mix the marrow in the bag by gentle to and fro motions. The marrow is now plasma reduced and is ready for infusion.

Other plasma reduction approaches:
- Cobe 2991 wash with heparanized saline
- Manual centrifugation and reconstitute with plasmalyte, 5 percent Human Serum Albumin or heparinized NaCl.
INDEX

A

ABO blood group system 2
Acid-base balance 166
Acute lymphoblastic leukemia 242
Acute myelogenous leukemia 241
Acute respiratory distress syndrome 153
Adenine nucleotide 92
Antifibrinolytic agents 222
Apoptosis 148
Appropriate transfusion 150
Assays for platelet antibodies 85
Autologous blood transfusion 22, 170

B

Bernard-Soulier syndrome 77
Bleeding disorders 188
Blood bank physicians 2
Blood banking research 1
Blood component administration 122
blood administration steps 126
blood warmers 127
immediate pre-infusion identification steps 127
infusion steps 128
infusion supplies and sets 127
post-infusion steps 129
laboratory testing 124
pre-surgical blood group typing and compatibility testing 124
red cell ABO typing and compatibility testing 124
thawing and pooling components 124
physician’s order and informed consent 123
recognition and management of reactions 129
auditing blood component transfusions 130
sample collection and labeling 123
special processing 125
release of blood components from the blood bank laboratory 125
Blood component transfusion guidelines 133
compatibility testing prior to transfusion 141
components with reduced CMV risk 140
cryoprecipitated antihemophilic factor 138
cryoprecipitate-reduced plasma 138
frozen deglycerolized red cells 135
frozen plasma 137
granulocyte concentrates 139
irradiated cellular blood components 140
leukocyte-reduced blood component 140
leukocyte-reduced blood components 139
platelet 135
adults and children 135, 136
in refractory patients 136
more than one dose per day 136
red blood cells 133
adults 134
infants and children 134
source of blood 142
washed red cells 135
Blood donation adverse events 30
accidental arterial puncture 32
donor injuries and reactions 30
iron depletion in the donor 32
nerve injury or irritation 32
postdonation hospitalization or death 33
vasovagal reaction 30
Blood donation process 30
Blood donations in the United States 27
Blood donor suitability 28
criteria for diseases, conditions, and behaviors 29
criteria for hemoglobin 29
criteria for vital signs and inspection of the arms 28
Blood group reagents and cells 16
Blood safety in developing countries 5
Blood transfusion in the operating room 159
blood product replacement protocol 161
guidelines for transfusion 159
problems related to the storage lesion 161
recognition of ABO incompatible transfusion 160
standard precautions 160
transfusion of blood components during surgery in infants and children 162
Bone marrow 278, 297
Bone marrow transplantation 294
ABO mismatched transplants 296
blood product support 298
choice of ABO group of blood products 296
coagulation studies 295
donor 294
platelet count 295
post-transplant transfusion support 299
pre-transplant work up 294
stem cell collection 297
stem cell infusion 297
transfusion support for the bone marrow harvest 295
venous access 295
Breast cancer 247

C

Cardiac disease 152
Cardiopulmonary bypass surgery 192
Chronic lymphocytic leukemia 243
Chronic myelogenous leukemia 242
Coagulation abnormalities 167
Coagulation proteins 186
Coagulation therapy 215
clinical application 217
complications 221
dosing and duration 217
evolution
hemophilia A 215
hemophilia B 216
management of patients with inhibitors 222
inhibitors in hemophilia B 224
suppression of inhibitors 224
treatment of acute hemorrhage 223
prophylactic coagulation factor replacement therapy 218
Component preparation from whole-blood donations 19
calibration of blood bank 20
free blood components 21
irradiated cellular blood components 21
leucocyte reduction of 21
optimal preparation 20
preparation by apheresis 21
quality control of blood components 20, 21
Congenital cellular immunodeficiency 114
Cryoprecipitate 210
administration and dosage 213
indications for use 211
Cryopreservation 281

D
Deferral rate 29
Desmopressin 221
Disorders of platelet function 76
Disposal of biohazardous waste 23
Disseminated intravascular coagulation 194
Drugs inhibiting platelet function 189

E
Enhanced red cell consumption 147
Erythropoietin 146
Evaluation of platelets 84

F
Factor VIII deficiency 212
Febrile nonhemolytic transfusion reaction 105
Fibrin sealant 212
Financial support issues for blood banks 25

G
Germ cell cancers 248
Glanzmann’s thrombasthenia 77
Graft-versus-host disease 50, 104
Granulocyte transfusions 115, 299

H
Hematopoietic stem cell processing 278
processing methods 279
quality assurance (QA) 281
quality control testing techniques 281
regulatory issues 281
routine methods 279
specialized methods 280
Hematopoietic stem cell transplant recipients 114
Hematopoietic stem cell transplantation 228
chemotherapeutic agents 232
patient eligibility 232
disease factors 232
host factors 232
scientific background 229
diseases treated 230
graft-versus-tumor effect 230
high-dose therapy rationale 229
indications 230
stem cell sources 231
total-body irradiation 232
allogeneic transplant 233
autologous transplant 235
preparative regimens 233
Hematopoietic stem cells 235
hematopoietic growth factors and cytokines 240
Hematopoietic stem cells 278
Hemoglobin color scale 9
Hemoglobin function 167
Hemophilia A 212
Hemorrhagic cystitis 299
Hemostasis 186
Heparin-associated thrombocytopenia 201
Hodgkin’s disease 115, 244
HPC cryopreservation 284
side effects 285
Hypersplenism 191
Hypofibrinogenemia 211
Hypotension and bleeding 152
Hypoxia 152

I
Idiopathic thrombocytopenic purpura 72
Immunosuppression 168
Impedance platelet aggregometry 67
Infusion of hematopoietic stem cells 288
Infusion of umbilical cord blood hematopoietic stem cells 290
Iron metabolism 147
Irradiation 111
adverse effects 112
dosage 112
indication 113, 114
absolute 114
controversial 117
general 113
probable 116
nonindications 118
possible alternatives 118
quality control and quality assurance 113
Irradiator units 111

L
Lactic acidosis 153
Leukoreduction of blood components 36
alloimmunization 41
bacterial contamination of blood components 49
fever and rigors in recipients 38
graft-versus-host disease 50
HLA antibodies and refractoriness to platelet transfusion 39
preventing cytomegalovirus transmission 42
infection with a second-strain of CMV 45
reactivation of latent CMV infection 45
transfusion associated immune suppression 46
cost and length of hospital stay 48
randomized clinical trials 47
Liver disease 192
Lumiaggregometry 67

M
Management of transfusion reactions 143
acute hemolytic transfusion reactions 143
Massive transfusion protocol 170
Multiple myeloma 245
Myelodysplastic syndrome 246
Myelofibrosis 246

N
National Marrow Donor Program® 256
NMDP cord blood program 257
obtaining an NMDP-facilitated stem cell transplant 258
research program 258
searching the NMDP registry 260
selection of donors/stem cell source 262
the NMDP registry 256
unrelated donor stem cell transplantation 258
Index 305

Neonatal alloimmune thrombocytopenia 106
Non-Hodgkin’s lymphoma 244
Nutritional deficiencies 147

O
Ovarian cancer 247
Oxygen delivery requirements 149

P
Pathogenesis of anemia 146
Pediatric critically III 154
Peripheral blood 278
Peripheral blood stem cells 297
Physiology of blood loss 165
Plasma product
- fresh frozen plasma 203
- frozen plasma 203
- liquid plasma 205
- methylene blue-treated 205
- plasma cryoprecipitate reduced 205
- solvent/detergent-treated plasma 205
- thawed plasma 203
Plasma products 204
Plasma transfusion therapy 203
Platelet activation 92
Platelet aggregation 92
Platelet biochemistry and function
- in vitro evaluation 88
- in vitro evaluation 89
Platelet function analyzers 84
Platelet function tests 84
Platelet preservation 90
- methods 93
- detection of platelet function 94
- device for monitoring 94
Platelet survival in circulation 90
Platelet transfusion dose 84
Platelet transfusion therapy
- apheresis 81
- concentrates 81
- cryopreserved 82
- lyophilized 82
- plasma products 83
Platelet transfusions 83
Platelets 64
- disorders 71
- function 65
- adherence 66
- aggregation 67
- secretion 66
- shape change 66
- membrane glycoproteins 68
- IIb/IIIa receptor 68
- P-selectin 71
- morphology
- light microscopy 64
- ultrastructure 64, 65
- new advances in measuring platelet function 67
- origin and development 65
- procoagulant activity 68
Postdonation tests 29

Q
Quality management program 23
- automation in blood banking 24
- documentation systems 23
- inventory control of consumables
- 25
- inventory management 25
- quality control program 23
- quality tools 24
- recordkeeping 24
- reporting of test results 24

R
Red cell
- ABO typing 124
- aging 147
- destruction 148
- production 146
- serology testing 15
- substitutes 169
Regional blood transfusion center 11
- blood collection 15
- donor recruitment and blood collection 13
- recruitment of voluntary donors 14
- donor screening and selection 14
- organization 12
- equipment and spares 13
- infrastructure requirements 13
- legal and regulatory authority 12
- planning 12
- staffing 13
- roles and responsibilities 11
Renal cell cancers 248
Renal disease 194
Reperfusion injury 51
Reporting transfusion reactions 144
Role of platelets 186

S
Single platelet counting 68
Solid organ transplantation 102
Solid tumors 246
Sonoclot 67
 Stem cell transplantation 102, 103
Storage lesion of blood components 166
Stored red cells 153

T
Template bleeding time 85
The HLA system 98, 259
- alloimmunization 103
- clinical HLA testing 100
- HLA antibody screening 101
- lymphocyte crossmatch 101
- molecular typing of HLA alleles 100
- serologic typing of HLA antigens 100
- disease association 106
- genomic organization of the human MHC 98
- HLA haplotypes 98
- human minor histocompatibility antigens 101
- parentage testing 106
- peptide presenting role 99
- structure and polymorphism 99
- tissue expression 99
- transfusion therapy 103
- transplantation 101
Thrombocytopenias 71, 72
- drug-induced 75
- gestational 74
- heparin-induced 75
- idiopathic thrombocytopenic purpura 72
- inherited 76
- Thromboelastography 67
- Thrombosis 198
- Thrombotic thrombocytopenic purpura 207
Total-body irradiation 232
Transfusing physician's responsibility 143
Transfusion in sickle cell disease 173
- alternatives to blood transfusion 178
- complications 177
- indications
- acute 174
- chronic 175
- equivocal 175
- infection 178
- nonindications 175

storage lesion of blood components 166
stored red cells 153

T
Template bleeding time 85
The HLA system 98, 259
- alloimmunization 103
- clinical HLA testing 100
- HLA antibody screening 101
- lymphocyte crossmatch 101
- molecular typing of HLA alleles 100
- serologic typing of HLA antigens 100
- disease association 106
- genomic organization of the human MHC 98
- HLA haplotypes 98
- human minor histocompatibility antigens 101
- parentage testing 106
- peptide presenting role 99
- structure and polymorphism 99
- tissue expression 99
- transfusion therapy 103
- transplantation 101
Thrombocytopenias 71, 72
- drug-induced 75
- gestational 74
- heparin-induced 75
- idiopathic thrombocytopenic purpura 72
- inherited 76
- Thromboelastography 67
- Thrombosis 198
- Thrombotic thrombocytopenic purpura 207
Total-body irradiation 232
Transfusing physician’s responsibility 143
Transfusion in sickle cell disease 173
- alternatives to blood transfusion 178
- complications 177
- indications
- acute 174
- chronic 175
- equivocal 175
- infection 178
- nonindications 175
recommended manual scheme 176
transfusion in beta-thalassemia major 179
adverse reactions 182
blood products for special patient populations 180
blood transfusion therapy 180
compatibility testing 181
transfusion regimens 181
transfusion methods 176
Transfusion medicine 1, 3
Transfusion-associated graft-versus-host disease 110
clinical manifestations 110
genetic documentation 111
treatment 111
irradiation of blood components 111
Transfusion-related acute lung injury 105
Transfusion-transmissible infections 16
hepatitis B virus 17
hepatitis C virus 17
human immunodeficiency virus 17
indirect sandwich ELISA for donor screening tests 17
malarial parasite 17
Transportation of blood components 22

U
Umbilical cord blood 279, 297
Umbilical cord blood banking 267
collection 270
ex utero collection method 271
liquid preservation of cord blood 271
donor recruitment 267
infectious disease screening 270
medical evaluation of the donor 268
medical history issues 269
medical history sources 268
obtaining the medical history 269
obtaining consent 267
processing, cryopreservation and storage 272
quality assurance and quality control 275
thawing and washing of umbilical cord blood units 273
transportation of cord blood 273, 274
Uremic bleeding 213

V
Vascular endothelium 188
Venous thromboembolism 198
prevention 199
treatment 200
Voluntary blood donation 9
von Willebrand’s disease 78, 211
acquired 81
classification 78
pseudo 81
testing 78
treatment 80

W
WHO blood safety program 6
additional WHO learning materials 8
blood cold chain project 8
costing blood transfusion services 6
distance learning material 8
evaluation and bulk procurement of HIV test kits 9
External Quality Assessment Schemes 7
Global Collaboration for Blood Safety 7
hemoglobin color scale 9
nationally-coordinated blood transfusion programs 6
WHO Quality Management Program 7
World blood donor day 9
1. Blood Safety 1
2. Donor Recruitment, Donor Screening, Blood Collection and Processing 5
3. Donor Selection and Blood Collection 14
4. Some Important Links Related to Blood Banking, Transfusion Medicine and Cellular and Molecular Therapies 18
5. Thrombotic Thrombocytopenic Purpura 22
“Blood Sustains Life”—Charaka Samhita 6th century AD, India. Blood is a precious life saving fluid with a variety of proteins and blood cells. Blood is one of the life saving gifts that one person can give to the other person in need. It cannot be manufactured. It is needed every day by mothers, who experience complications during child birth, children suffering from clinical complications, victims of accidents, patients needing surgery and transfusions. Good blood saves life. But, contaminated blood causes diseases in thousands of people in the developing countries including Hepatitis B and C, HIV, Chagas’ disease, malaria, syphilis and other chronic diseases. With the help of several Rotary Clubs, a few blood banks and transfusion medicine staff, we have launched a safe blood initiative. This initiative aims at improving the quality of blood and blood products, so that every person receiving a transfusion of blood or blood products can count on the blood being free of life-threatening pathogens.

Blood bank and transfusion medicine staff support and cooperation can help to:

- Recruit healthy volunteer blood donation through an intensive public awareness campaign.
- Train blood technicians and clinical leaders in proper techniques for blood screening and handling.
- Provide standard operating procedures (SOPs) and accurate testing kits to blood bank staff.
- Establish quality control programs among all blood banks in the developing countries.
- Establish “regional blood banks and transfusion medicine centers” as model, so that they provide the needed training to other blood bank and transfusion medicine staff.

On April 7, 2000, the WHO launched a new blood-safety campaign, which aims to increase the availability of safe blood in developing countries. The organization issued facts and figures on the state of the world’s blood supply to spur the governments to establish national transfusion systems. The cornerstone of a safe and adequate supply of blood and blood products is the recruitment, selection and retentions of voluntary, non-remunerated blood donors from low risk populations.

WGO has developed a set of simple guidelines designed to assist those responsible for blood donor recruitment in resource poor settings to develop and implement a program to improve communication with blood donors. These guidelines provide approaches for setting up a communication program — organizing, collecting information, and developing plans; as well as provide ideas for recruiting, educating and retaining safe donors that individual centers might consider. Despite this, family/replacement and paid donors, which are associated with a significantly higher prevalence of transfusion transmissible infections (TTIs) such as HIV, Hepatitis C, Hepatitis B, Syphilis, Malaria and Chagas disease, still make up over 50 percent of blood donations in the developing countries.

The WHO strategy for blood safety recommends that all donated blood be tested for HIV, Hepatitis B and C, syphilis and, where appropriate, other markers of infection such as Chagas disease and HTLV1/11. According to WHO of the 81 million units collected annually, up to 6 million units are not screened for all relevant TTIs, mainly in developing countries. Twenty-five percent of the countries also reported that blood has been issued without testing due to lack of test kits. Therefore, millions of patients who are transfused with untreated blood are at risk for transfusion-transmitted infections. Global Data Base on Blood Safety (GDBS) data indicates that the use of whole blood is 15 times higher in developing countries than in developed world, resulting in inadequate provision of the life-saving support for patients requiring specialized treatment with blood component therapy.
Blood Transfusion Safety

National blood donor program for the education, recruitment and retention of low-risk blood donors, including community-based voluntary blood donor organizations and youth program;

• Appointment of an officer responsible for the national blood donor program to include donor education, motivation, recruitment and retention;
• Training of donor recruitment staff in donor education, motivation, recruitment, selection and retention;
• Identification of donor populations at low risk for TTIs and strategies for behavioral changes;
• Development of donor education and recruitment material;
• Educational and media campaigns in workplaces, communities and educational institutions;
• Establishment and maintenance of a database/register of donor records;
• Guidelines and protocols for donor selection and deferral, donor confidentiality and donor care;
• Guidelines on the management of donor sessions and blood collection
• Monitoring of TTIs in donor population;
• Training of staff in pre- and post-donation counseling;
• Donor notification and referral counseling;
• Monitoring and evaluation of blood donor program.

World Blood Donor Day (WBDD) will focus on the youth programs, building on the success of the World Health Day and the theme “Blood Save Lives, Safe Blood Starts with me”.

Safe Blood starts with Me

Western Province Blood Transfusion Service of South Africa have prepared blood safety program based on WHO recommendations:

According to them, commitment to safeguard the blood supply rests on multiple layers of protections, not just blood tests. Their goal is to reduce the transfusion transmitted infections to as low a level as possible.

Following measures form part of the standards and Principals of transfusion practice throughout the country:

• A voluntary donor base: This has been shown to be the source of the safest blood.
• Education of the prospective donor: Presentations and literature handouts for new donor clinics, Educational material for new and current donors on safe blood donation.
• Pre-donation screening: Screening of donors by means of indirect and direct questioning, about health and at risk behavior.
• Testing: Only licensed and approved tests are used for both initial screening and confirmatory testing of repeatedly reactive samples.
• Sterilization of blood products where feasible: Many of the plasma derived products are subjected to approved sterilization procedures Factor 8, Factor 9, immunoglobulins, stabilized serum; albumin and some FFP units are virally inactivated.

Clinicians may also avail themselves of alternatives to allogenic blood:
• Patients may need to have autologous or designated blood reserved for their operation
• Intraoperative salvage or preoperative hemodilution
• Use of non-plasma derivative volume expanders.

Dr. Neelam Dhingra and associates at the WHO have launched global blood safety initiatives. We summarize few salient points from their recommendations.

Quality Systems in Blood Transfusion Service

At least following elements should be in place: a national quality system, full-time dedicated staff, standard operating procedures, quality assurance, program for blood screening and participation in an external quality assessment schemes. GDBS data shows globally only 16 percent of the countries have all of these elements fully in place with more than half of the percentage being made up of developed countries.

Training in Blood Transfusion Service

Blood transfusion services require a comprehensive, multidisciplinary approach to training for all BTS personnel, including donors recruitment and blood collection staff, medical officers, quality managers, and clinicians who actually prescribe transfusions.
WHO Strategies for Blood Safety

WHO has launched blood safety as one of its priorities and the World Health Day 2000 was dedicated to the theme of blood safety. Blood transfusion services require an adequate and sustainable budget, a national policy and plan supported by a legislative framework with regulations that govern all activities.

There is a need to coordinate efforts at national, regional and global level and develop effective collaboration with experts, institutions, organizations and other partners working for blood safety at different levels. WHO can provide the following support to countries in providing standards, recommendations, guidelines, advocacy, technical guidance, capacity building – human resource development, training and infrastructure development of organizational and quality systems?

WHO recommends the following integrated strategy to national health authorities to ensure safe and accessible blood:

- the establishment of well-organized, nationally-coordinated blood transfusion services to ensure the timely availability of safe blood and blood products for all patients requiring transfusion;
- the collection of blood only from voluntary non-remunerated blood donors from low-risk populations;
- testing for transfusion-transmissible infections, blood grouping and compatibility testing;
- the safe and appropriate use of blood and reduction in unnecessary transfusion;
- quality systems covering the entire transfusion process, from donor recruitment to the follow-up of the recipients of transfusion.

Program Areas

- HIV counseling and testing;
- Prevention of medical transmission of HIV;
- Prevention and care of sexually transmitted infections;
- Most at-risk populations;
- Public-private partnerships;
- Behavior change, communications.

Care and Treatment

CDC supports country efforts to strengthen and expand care, support and treatment option for people suffering from HIV/AIDS and opportunistic infections. These five strategies build on the strengthening of communities to provide option ranging from home-based to clinical care and social support.

- Tuberculosis prevention and care;
- Prevention and treatment of opportunistic infections;
- Palliative care;
- Antiretroviral therapy;
- Preventing mother-to-child HIV transmission.

Surveillance and Infrastructure

CDC supports countries to strengthen their capacity and develop infrastructure to manage, implement and evaluate national HIV/AIDS programs and to monitor in the epidemic. CDC has identified the following areas of importance.

- Surveillance
- Laboratory capacity building
- Informatics
- Monitoring and evaluation
- Training.

GAP Programs Make a Difference all over the World

In India

- HIV/AIDS training improved infection control and empowered nurses
- A new community care model is bringing support for families “close to home”

In Boswana

- Radio program entertains while educating local listeners about how to reduce HIV risk.
In Kenya

- New center provides HIV testing and counseling for Kenya’s deaf citizens.

In Malawi

- A new touch screen electronic patient monitoring system is improving patient care and data collection.

Around the World

- Generic training package helps countries reduce mother-to-child transmission of HIV and improve the health of pregnant women and infants.

International Laboratory-related Resources and Activity Directory

This directory links to training materials, guidelines, manuals and resources developed by CDC and partner organizations to promote laboratory practices in global laboratory setting. 
http://www.phppo.cdc.gov/dls/ila

Information/Toolboxes

- Laboratory assessment tools
  — WHO lab assessment tools
- Laboratory training
  — Training facilitators information
- HIV/AIDS
- Laboratory quality systems
- Laboratory safety
  — WHO laboratory biosafety manual
  — CDC/NIH biosafety microbiological and medical laboratories
- Tuberculosis (TB)
- Malaria/Parasitology
- Hematology/clinical chemistry
- CD4/Viral load.
In the recent issue of transfusion (45:2005), Owusu-Ofori and associates describe the use of rapid tests to screen potential blood donors for transfusion-transmitted diseases. The authors report on the pre-donation testing of potential blood donors for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B surface antigen before blood donation (45:133-140,2005). Blood was subsequently collected only from donors who screened negative; pooled post-donation plasma samples were tested for HIV, hepatitis B virus (HBV), and HCV by nucleic acid amplification methods.

In developing countries, the transmission of HIV and other blood-borne pathogens via blood transfusion is largely preventable by the establishment of nationally regulated, regional blood transfusion systems that provide an adequate supply of safe blood and through the elimination of unnecessary transfusions. The development of such regional systems, however, has been hampered by severe financial resource limitations. According to WHO, only 90 percent of the blood collected in Africa are screened for HIV, 55 percent for HBV and only 40 percent for HCV.

According to an editorial in the same journal, Transfusion (45:131,2005), although there are little data available on rates of adverse transfusion events in developing countries, the combination of low technologist skill level and inadequate quality systems is of great concern. Blood transfusion practice is a complex area of medical care that carries potentially serious consequences when not practiced carefully. Even in developed countries with adequate resources, adverse events are not infrequent in transfusion practice. Sustainable good-quality blood transfusion systems can only be achieved with national oversight and a commitment to safe blood transfusion as a part of the health care system.

Despite the efforts made in recent years to improve blood safety in African countries, the situation is still worrying. Among 46 Member States of the African region, only 13 have implemented their National Policy and that 25 percent of blood transfused in the Region, is still not tested for HIV, and this figure is higher for Hepatitis B, Hepatitis C and syphilis (Report of a workshop at HARARE May2-5, 2000). Regional Office of the Member States, in collaboration with WHO, has come up with following suggestions.

Objectives
- Situation of transfusion safety in the participating countries assessed and updated.
- Priority measures to improve blood transfusion service in the region identified.
- Framework for implementing regional blood transfusion quality assurance program defined.

According to the experts the prerequisites of a safe blood initiative are the same regardless of the region or country and these are:
- Government commitment to blood safety;
- Sound organizational structures;
- Safe blood donors;
- Quality in blood screening, storage, transportation and distribution;
- Appropriate use of blood and blood products.

In the last AAPI conference, the Minister of Health, Government of India, proclaimed that the top priority of his Government is the “Safe Blood Initiative”.

Donor Recruitment, Donor Screening, Blood Collection and Processing
The constraints to quality in national blood initiatives in India are as follows:"
- Lack of national blood policy or statutes;
- Delegation of adequate regulatory responsibilities for agencies involved in quality assurance;
- Blood programs not nationally coordinated;
- Limited knowledge on quality management;
- Inadequate blood supplies;
- Lack of training programs in transfusion medicine.

The following efforts can be initiated immediately by regional Blood Transfusion Centers without having to wait for a national policy or establishment of national blood transfusion service.

Increase public education on Safe Blood Initiative
- Target low risk groups;
- Establish net-work;
- Motivate blood donors;
- Establish donor records;
- Provide good customer service;
- Perfect donor selection criteria;
- Pre-and-post donation counseling;
- Ensure blood collection teams adhere to established standards;
- Develop and implement SOPs in all aspects of blood transfusion;
- Ensure that all personnel are trained for the jobs they are doing;
- Ensure internal quality control;
- Check conformation of results;
- Pay attention to the choice and evaluation of test kits;
- Use established procedures for the preparation of blood components.
- Establish continuing education;
- Introduce at least one external quality assessment scheme.

Safe transfusion therapy is a basic need for better health care. Therefore, it is essential to distinguish transfusion safety from blood safety. Blood safety refers to the safety of the product. Whereas, transfusion safety refers to the safety of the overall process of transfusion, from donor to recipient.

Non-infectious serious hazards of transfusion are described as follows by Dr. Suny Dzik in Blood News May 2002:

Mis-trasfusion of blood: failure to give “the right blood or blood-product to the right patient at the right time for the right reason”. Transfusion-related acute lung injury (TRALI): Immune-mediated lung injury. Other transfusion-related hazards include: circulatory overload and cardiac toxicity, metabolic risk to neonates.

Some useful references for Blood Safety Initiatives include:
- American Association of Blood Banks http://www.aabb.org/professional-development/computer-assisted-conference...
- Center for Disease Control and Prevention http://www.cdc.gov/nchstp/od/gap/strategies/2-3-blood-safety.htm
- Canadian Blood Services http://www.bloodservices.ca
- http://www.who.int/bloodsafety/

DONOR SELECTION AND BLOOD COLLECTION

General Principles and Standards

1. The blood donation area should be well lit, adequately ventilated, clean and attractive.
2. The donor should be attended to promptly. Any unavoidable delay should be explained to the donor in advance.
3. The staff should be professional as well as friendly and ensure that the blood donation is made a pleasant experience.
4. All prospective allogeneic, autologous or apheresis blood donors must be given educational material regarding the infectious diseases transmissible by blood transfusion, and the opportunity for self-deferral.
5. All prospective blood or apheresis donors must be given educational material regarding the adverse reactions that can occur after blood donation/apheresis.
6. The blood bank should obtain the informed-consent from the allogeneic, autologous or apheresis blood donor
7. The medical history and examination must be performed as per SOP by a suitably qualified person prior to blood donation - to ascertain donor suitability.
8. All material for blood collection and transfusion shall be sterile, pyrogen free and disposable.

**DONOR RECRUITMENT, SCREENING AND SELECTION**

Blood donors can be classified as:
1. Voluntary non remunerated donors (VNRD), who give blood of their own free will with a purely altruistic motive to help an unknown person and not for payment or any favors. They respond readily to appeals for blood donation and also donate blood regularly. Since they are not under duress to donate, they will give reliable information at the time of donor screening and therefore, there is a lower incidence of TTI among voluntary donors.

2. Replacement donors, who donate to replace blood issued by the blood center to their relatives or friends.

3. Directed donors who donate blood to a specific, named patient and designated donors who designate their donations for a particular patient's recurrent blood requirement.

4. Autologous donors who donate for their own use.

5. Paid/professional donors who give blood in return for payment or other favors. The quality of this blood is poor and world over it has been proved that there is a higher incidence of transfusion associated infections among them. Paid donations are banned in India since 1998 as in most other countries.

The first step in ensuring blood safety is getting a safe donor. This involves:

I. Motivation and recruitment of voluntary donors and recall and retention of safe, voluntary, repeat, blood donors

II. Donor screening and selection

III. Donor deferral registry.

**Motivation and Recruitment of Voluntary Donors and Recall and Retention of Safe, Voluntary, Repeat, Blood Donors**

An effective and safe blood transfusion service requires a program aimed at motivation, recruitment and retention of voluntary non-remunerated, regular blood donors; minimizing replacement donation; increasing autologous donations and elimination of paid donors altogether.

The donation is considered voluntary non-remunerated blood donation if a person donates whole blood, plasma or cellular components by his/her own free will, receiving no payment in cash, or in kind which could be considered a substitute for money, including time off from work other than the time required for donation.

**Donor Screening and Selection**

In selecting individuals for blood donation the main purpose is to determine whether the person is in good health in order to prevent.

1. Any harm to the donor’s own health.

2. Transmission of diseases that could be detrimental to the health of the recipient.

**Registration:** This is the first step in the selection process and is done to get the donor demographic and contact details for proper identification, traceability and recall. It should include the name, sex, age, date of birth, contact addresses and phone numbers, date of last donation.

**Predonation information:** Next he/she is given the necessary predonation information to explain the donation process; potential post-donation reactions and post-donation care; what happens to the blood collected, i.e. testing, processing etc. how it is used.

**Opportunity for self-exclusion or self-deferral:** The predonation information explain who can donate (Donor eligibility criteria) and who cannot (Donor Deferral Criteria) with special emphasis on the risk behavior and factors that could lead to Transfusion Transmissible Infections. Donors are thus provided the opportunity for self-exclusion or self-deferral. In addition, an interview and counseling also helps the person to self-defer if there are risk factors that can harm him/her or the recipient.

**Donor questionnaire and informed consent:** Next the donor has to fill the Questionnaire, which is both a tool for risk behavior/ risk factor assessment as well as an important legal document bearing the signature of the donor.

**Medical history and examination:** This is the final step in donor selection that is carried out by a qualified physician/BTS staff to determine suitability to donate based on “donor eligibility and deferral criteria”.


Deferral can be either permanent or temporary. These criteria and time of deferral vary in different countries. The details of deferral are to be maintained by the blood center.

A complete medical and physical examination of blood donors is generally not possible in practice. One has to rely on donor’s answers in the donor questionnaire regarding his or her medical history and general health, combined with a simple inspection of the donor’s appearance, and a limited physical and laboratory examinations.

**Criteria for Protection of Donor**

1. General appearance – the prospective donor should appear to be in good health and should be deferred if they appear ill, under the influence of drugs/alcohol or even very nervous.
2. The donor should not be fasting (should have had a meal not more than 4 hours ago). The donation should not be accepted within 1 hour of a heavy meal.
3. Age-minimum – 18 years and maximum – 60 years.
4. Interval between two whole blood donations of 350/450 ml should not be less than 90 days. Whole blood donation after hemapheresis/cytapheresis should be deferred for at least 48 hours.
5. Quantity of blood collected – a standard donation is 450ml + / - 10 percent exclusive of anticoagulant solution.
6. Weight - should be more than 50 kg to collect 450 ml and between 45 and 55 kg to collect 350 ml blood. No more than 13 percent of the estimated blood volume should be collected as whole blood during one blood donation.
7. Blood pressure – should be within normal limits (systolic not more than 160 and diastolic not more than 100 mm of Hg) without medication.
8. Pulse – should be regular, between 60 to 100 beats per minute except in athlete where a lower pulse rate (40 to 60 beats) may be acceptable.
9. Temperature – Oral temperature shall be 37.5° C +/- 0.2° C (99.5°F +/- 0.5°F)
10. Hemoglobin – should be determined each time before donation and shall not be less than 12.5 gm/dl.
11. Hazardous occupation – Donors with hazardous occupations or hobbies (pilots, bus or train drivers, crane operators, persons working at a height on scaffolding or ladders, gliding, diving, mountain climbing etc.) should be advised to wait for 12 hours before returning to their occupation or hobby.
12. Medical history
   - History of cancer, autoimmune disorders, epilepsy; disorders of liver, lung, heart, and kidney; bleeding disorders, polycythemia rubra vera and hypertension should be permanently deferred from donating blood.
   - Asthma – potential donors who are asthmatic can donate, if asymptomatic without treatment on the day of donation.
   - Diabetes mellitus – People with diabetes under control with oral antidiabetic drugs, can donate blood. Insulin dependent diabetics shall be permanently deferred.
   - Bronchitis – Persons with symptoms of severe, chronic bronchitis should not be accepted as donors.
   - Persons with this condition should be rejected.
   - Surgical procedure – donors should be deferred for 1 year after surgery or 6 months after recovery from a surgery.
   - Weight loss – unexplained excessive weight loss (more than 10 kg in a month) without obvious reasons in a prospective donor could indicate an undiagnose serious illness and donor suitability should be evaluated by the physician.
   - Women – pregnant women shall not donate blood till 6 months after delivery or abortion; during lactation and during menstrual periods.

**Criteria for Protection of the Recipient**

1. **Infectious diseases** – a prospective donor shall be free from infectious diseases known to be transmissible by blood in so far as can be determined by the questionnaire, history and examination.
   - Blood transfusion – prospective donors who have received whole blood, blood component or blood derivatives should be deferred for 6 months.
   - Jaundice and hepatitis – persons giving history of viral hepatitis or close contact with a patient
should be deferred for one year. They can be accepted as blood donors after one year provided the test for HBsAg and anti-HCV are negative. Persons ever tested positive for Hepatitis B or C should be deferred permanently.

- **AIDS** – All donors should be given awareness and/or educational material regarding AIDS and how HIV spreads to enable persons with risk behavior defer from donating blood. Donor questionnaire must include questions relating to the risk behavior for HIV and persons admitting to such behavior should be deferred permanently. It must also include signs and symptoms of HIV infection and AIDS, and answers suggestive of the disease must be evaluated for determining donor suitability. These will include H/o unexplained fever, weight loss greater than 10 percent of the body weight in one month, headache, and white patches in the mouth.

- **Sexually transmitted diseases** – Prospective donors giving history of syphilis, gonorrhea or other STDs or treatment for the same shall be deferred permanently.

- **Tuberculosis** – Prospective donors giving history of tuberculosis or treatment for the same shall be deferred permanently.

- **Typhoid** – one year after recovery

- **Malaria** – Persons with a history of malaria should be deferred for 3 months in endemic areas and 3 years in non-endemic areas.

- **Skin infections** – Persons with skin infections should not be accepted as blood donors.

- **Venipuncture site** – the skin at the venipuncture site shall be free of lesions.

2. **Immunization and Vaccination**

- **Cholera, Hepatitis B, diphtheria, typhoid, polio injection and tetanus toxoid** which are based on Toxins and killed virus, bacteria or rickettsia – donor is acceptable if asymptomatic and afebrile.

- **Animal bite and rabies vaccination** – Persons with history of animal bite shall be deferred for 1 year after the bite whether or not treated with antirabies vaccine.

- **German measles** – defer for 6 weeks.

- **Hepatitis a Immune globulin** – defer for 1 year

- **Measles, mumps, oral polio or yellow fever** that are attenuated virus vaccines – defer for 2 weeks.

- **Passive immunization with animal serum product for tetanus, rabies, snake bite, gas gangrene** – defer for 6 weeks.

**Drug Therapy**

Persons on drug therapy, including antibiotics may have a clinical condition could be deleterious to the donor or to the recipient. Therefore, they should be evaluated to determined donor suitability. Persons who have taken accutane (for acne) should be deferred for 30 days.

Persons who have been treated with Tegison (for psoriasis) should be permanently deferred.

**Acceptable Drugs**

The following common prescription and over the counter drugs are not a reason for donor deferral, unless there is an associated or underlying medical condition which makes the person unsuitable for donating blood.

- **Analgesics:** Unless it is for treatment of fever, possible infections or for therapy of collagen or vascular diseases

- **Aspirin:** Blood from a donor who gives history of aspirin ingestion cannot be used as the only source for platelet preparation, unless no aspirin has been ingested by the donor for a period of 5 days prior to donations.

- **Alcohol and parenteral drugs:** Obvious stigmata of drug or alcohol addiction shall exclude a prospective donor. Both arms must be inspected for evidence of for parenteral drug use.

**Donor Deferral Registry**

The blood bank should maintain a list of donors deferred earlier and the reasons for the deferral.

Donors deferred temporarily should be explained the reason for deferral and when they can donate again.

Notification of test results – Medical officer of the blood bank shall be responsible for a mechanism to notify the donors of all clinically significant abnormalities detected during the pre-donation evaluation or during laboratory testing.
All blood donors found to have a confirmed marker for HIV, Hepatitis B or C should be informed, as a part of a full counseling procedure, that they should not give further donations.

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5. All prospective blood or apheresis donors, must be given educational material regarding the adverse reactions that can occur after blood donation/apheresis.
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2. Replacement donors, who donate to replace blood issued by the blood center to their relatives or friends.
3. Directed donors who donate blood to a specific, named patient and designated donors who designate their donations for a particular patient’s recurrent blood requirement.
4. Autologous donors who donate for their own use.
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The first step in ensuring blood safety is getting a safe donor. This involves:

I. Motivation and recruitment of voluntary donors and recall and retention of safe, voluntary, repeat, blood donors.
II. Donor screening and selection.
III. Donor deferral registry.

Motivation and Recruitment of Voluntary Donors and Recall and Retention of Safe, Voluntary, Repeat, Blood donors

An effective and safe blood transfusion service requires a program aimed at motivation, recruitment and retention of voluntary non-remunerated, regular blood donors; minimizing replacement donation; increasing autologous donations and elimination of paid donors altogether.

The donation is considered voluntary non-remunerated blood donation if a person donates whole blood, plasma or cellular components by his/her own free will, receiving no payment in cash, or in kind which could be considered a substitute for money, including time off from work other than the time required for donation.

Donor Screening and Selection

In selecting individuals for blood donation the main purpose is to determine whether the person is in good health in order to prevent:

- Any harm to the donor’s own health
- Transmission of diseases that could be detrimental to the health of the recipient.
Registration

This is the first step in the selection process and is done to get the donor demographic and contact details for proper identification, traceability and recall. It should include the name, sex, age, date of birth, contact addresses and phone numbers, date of last donation.

Pre-donation Information

Next he/she is given the necessary pre-donation information to explain the donation process; potential post-donation reactions and post-donation care; what happens to the blood collected, i.e. testing, processing, etc. how it is used.

Opportunity for Self-exclusion or Self-deferral

The pre-donation information explain who can donate (Donor eligibility criteria) and who cannot (Donor deferral criteria) with special emphasis on the risk behavior and factors that could lead to transfusion Transmissible Infections. Donors are thus provided the opportunity for self-exclusion or self-deferral. In addition, an interview and counseling also helps the person to self-defer if there are risk factors that can harm him/her or the recipient.

Donor Questionnaire and Informed Consent

Next the donor has to fill the Questionnaire, which is both a tool for risk behavior/risk factor assessment as well as an important legal document bearing the signature of the donor.

Medical History and Examination

This is the final step in donor selection that is carried out by a qualified physician/BTS staff to determine suitability to donate based on “Donor eligibility and deferral criteria”. Deferral can be either permanent or temporary. These criteria and time of deferral vary in different countries. The details of deferral are to be maintained by the blood center.

A complete medical and physical examination of blood donors is generally not possible in practice. One has to rely on donor’s answers in the donor Questionnaire regarding his or her medical history and general health, combined with a simple inspection of the donor’s appearance, and a limited physical and laboratory examinations.

Criteria for Protection of Donor

1. General appearance – the prospective donor should appear to be in good health and should be deferred if they appear ill, under the influence of drugs/alcohol or even very nervous.
2. The donor should not be fasting (should have had a meal not more than 4 hours ago). The donation should not be accepted within 1 hour of a heavy meal.
3. Age-minimum – 18 years and maximum – 60 years.
4. Interval between two whole blood donations of 350/450 ml should not be less than 90 days. Whole blood donation after hemapheresis/cytapheresis should be deferred for at least 48 hours.
5. Quantity of blood collected – A standard donation is 450 ml +/- 10 percent exclusive of anticoagulant solution.
6. Weight – should be more than 50 kg to collect 450 ml and between 45 and 55 kg to collect 350 ml blood. No more than 13 percent of the estimated blood volume should be collected as whole blood during one blood donation.
7. Blood pressure – should be within normal limits (systolic not more than 160 and diastolic not more than 100 mm of Hg) without medication.
8. Pulse – should be regular, between 60 to 100 beats per minute except in athlete where a lower pulse rate (40 to 60 beats) may be acceptable.
9. Temperature – oral temperature shall be 37.5°C +/- 0.2°C (98.6°F +/- 0.5°F)
10. Hemoglobin – should be determined each time before donation and shall not be less than 12.5 gm/dl
11. Hazardous occupation – donors with hazardous occupations or hobbies (pilots, bus or train drivers, crane operators, persons working at a height on scaffolding or ladders, gliding, diving, mountain climbing etc.) should be advised to wait for 12 hours before returning to their occupation or hobby.
12. Medical History
   • History of cancer, autoimmune disorders, epilepsy; disorders of liver, lung, heart, and kidney; bleeding disorders, polycythemia rubra vera and hypertension should be permanently deferred from donating blood.
• Asthma – potential donors who are asthmatic can donate if asymptomatic without treatment on the day of donation.
• Diabetes mellitus – people with diabetes under control with oral antidiabetic drugs can donate blood. Insulin dependent diabetics shall be permanently deferred.
• Bronchitis – persons with symptoms of severe, chronic bronchitis should not be accepted as donors.
• Persons with this condition should be rejected.
• Surgical procedure – donors should be deferred for 1 year after surgery or 6 months after recovery from a surgery.
• Weight loss – unexplained excessive weight loss (more than 10 kg in a month) without obvious reasons in a prospective donor could indicate an undiagnosed serious illness and donor suitability should be evaluated by the physician.
• Women – pregnant women shall not donate blood till 6 months after delivery or abortion; during lactation and during menstrual periods.

Criteria for Protection of the Recipient

1. Infectious diseases – A prospective donor shall be free from infectious diseases known to be transmissible by blood in so far as can be determined by the questionnaire, history and examination.
   • Blood transfusion – prospective donors who have received whole blood, blood component or blood derivatives should be deferred for 6 months.
   • Jaundice and hepatitis - persons giving history of viral hepatitis or close contact with a patient should be deferred for one year. They can be accepted as blood donors after one year provided the test for HBsAg and anti-HCV are negative. Persons ever tested positive for hepatitis B or C should be deferred permanently.
   • AIDS – all donors should be given awareness and/or educational material regarding AIDS and how HIV spreads to enable persons with risk behavior defer from donating blood. Donor questionnaire must include questions relating to the risk behavior for HIV and persons admitting to such behavior should be deferred permanently. It must also include signs and symptoms of HIV infection and AIDS, and answers suggestive of the disease must be evaluated for determining donor suitability. These will include H/o unexplained fever, weight loss greater than 10 percent of the body weight in one month, headache, white patches in the mouth.
   • Sexually transmitted diseases – prospective donors giving history of syphilis, gonorrhea or other STDs or treatment for the same shall be deferred permanently.
   • Tuberculosis – prospective donors giving history of tuberculosis or treatment for the same shall be deferred permanently.
   • Typhoid – one year after recovery
   • Malaria – persons with a history of malaria should be deferred for 3 months in endemic areas and 3 years in non-endemic areas.
   • Skin infections – persons with skin infections should not be accepted as blood donors.
   • Venipuncture site - the skin at the venipuncture site shall be free of lesions.

2. Immunization and Vaccination
   • Cholera, hepatitis B, diphtheria, typhoid, polio injection and tetanus toxoid which are based on toxins and killed virus, bacteria or rickettsia – donor is acceptable if asymptomatic and afebrile
   • Animal bite and rabies vaccination – persons with history of animal bite shall be deferred for 1 year after the bite whether or not treated with antirabies vaccine.
   • German measles – defer for 6 weeks.
   • Hepatitis A immune globulin — defer for 1 year
   • Measles, mumps, oral polio or yellow fever that are attenuated virus vaccines – defer for 2 weeks.
   • Passive immunization with animal serum product for tetanus, rabies, snake bite, gas gangrene – defer for 6 weeks.

Drug Therapy

Persons on drug therapy, including antibiotics may have a clinical condition could be deleterious to the donor or to the recipient. Therefore, they should be
evaluated to determine donor suitability. Persons who have taken accutane (for acne) should be deferred for 30 days.

Persons who have been treated with Tegison (for psoriasis) should be permanently deferred.

**Acceptable Drugs**

The following common prescription and over-the-counter drugs are not a reason for donor deferral, unless there is an associated or underlying medical condition which makes the person unsuitable for donating blood.

- **Analgesics:** Unless it is for treatment of fever, possible infections or for therapy of collagen or vascular diseases
- **Aspirin:** Blood from a donor who gives history of aspirin ingestion cannot be used as the only source for platelet preparation, unless no aspirin has been ingested by the donor for a period of 5 days prior to donations.
- **Alcohol and parenteral drugs:** Obvious stigmata of drug or alcohol addiction shall exclude a prospective donor. Both arms must be inspected for evidence of parenteral drug use.

**Donor Deferral Registry**

The blood bank should maintain a list of donors deferred earlier and the reasons for the deferral.

Donors deferred temporarily should be explained the reason for deferral and when they can donate again.

**Notification of test results:** Medical officer of the blood bank shall be responsible for a mechanism to notify the donors of all clinically significant abnormalities detected during the pre-donation evaluation or during laboratory testing.

All blood donors found to have a confirmed marker for HIV, Hepatitis B or C should be informed, as a part of a full counseling procedure, that they should not give further donations.

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**Flow Chart 2.1: Blood donation**

- Address donor by name
  - Tally name on donor form
- Not pass
  - Recheck records
- Pass
  - Put bar code on blood bags, donor form and pilot tube
  - Collect blood
  - Documentation
    - Blood bag
    - Pilot tubes
    - Component preparation and storage
    - TTI screening
    - ABO and Rh
General Principles and Standards

1. The blood donation area should be well lit, adequately ventilated, clean and attractive.
2. The donor should be attended to promptly. Any unavoidable delay should be explained to the donor in advance.
3. The staff should be professional as well as friendly and ensure that the blood donation is made a pleasant experience.
4. All prospective allogeneic, autologous or apheresis blood donors must be given educational material regarding the infectious diseases transmissible by blood transfusion, and the opportunity for self-deferral.
5. All prospective blood or apheresis donors must be given educational material regarding the adverse reactions that can occur after blood donation/apheresis.
6. The blood bank should obtain the informed-consent consent from the allogeneic, autologous or apheresis blood donor.
7. The medical history and examination must be performed as per SOP by a suitably qualified person prior to blood donation - to ascertain donor suitability.
8. All material for blood collection and transfusion shall be sterile, pyrogen-free and disposable.

DONOR RECRUITMENT, SCREENING AND SELECTION

Blood donors can be classified as:

1. Voluntary non-remunerated donors (VNRD), who give blood of their own free will with a purely altruistic motive to help an unknown person and not for payment or any favors. They respond readily to appeals for blood donation and also donate blood regularly. Since they are not under duress to donate, they will give reliable information at the time of donor screening and therefore there is a lower incidence of TTI among voluntary donors.
2. Replacement donors, who donate to replace blood issued by the blood center to their relatives or friends
3. Directed donors who donate blood to a specific, named patient and Designated donors who designate their donations for a particular patient’s recurrent blood requirement
4. Autologous donors who donate for their own use.
5. Paid/professional donors who give blood in return for payment or other favors. The quality of this blood is poor and world over it has been proved that there is a higher incidence of transfusion associated infections among them. Paid donations are banned in India since 1998 as in most other countries.

The first step in ensuring blood safety is getting a safe donor. This involves:

I. Motivation and recruitment of voluntary donors and recall and retention of safe, voluntary, repeat blood donors.
II. Donor screening and selection.
III. Donor deferral registry.

Motivation and Recruitment of Voluntary Donors and Recall and Retention of Safe, Voluntary, Repeat, Blood Donors

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2. Transmission of diseases that could be detrimental to the health of the recipient.

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Donor Selection and Blood Collection

• German measles - defer for 6 weeks.
• Hepatitis A Immune Globulin - defer for 1 year
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Flow Chart 3.1: Blood donation
Some Important Links
Related to Blood Banking,
Transfusion Medicine and
Cellular and Molecular Therapies

American Association of Blood Banks

www.aabb.org
Education/Training Materials:
AABB offers a wide variety of education and training materials
Web-based Courses
a. Training manuals
b. Slide presentations
Web-based course are fee-based for the personal user and for the industrial users.

Training Manuals
These manuals are available free of charge.

Donor Services Training
Provides everything you need to know to process donors.
Orientation to the facility and blood banking - Requirements of regulatory agencies and accreditation organizations – overview of blood and blood components – donor Selection – collection of the blood from the donor – special procedures.

Primer for Blood Administration
Excellent Source for Developing Training Programs.
Introduction to Transfusion-Pretransfusion Activities- Identification and Safety-Role of Blood Bank-Blood Administration Practices.

Slide Presentations
Indications for platelet transfusion – Informed consent for blood transfusion – Serologic Evaluation of Patients with reactive autoantibodies-Transfusion consideration for ABO incompetent hematopoietic progenitor cell (HPC) transplants.

American Red Cross

www.redcross.org
Official web site. National Site
Look for links related to blood, marrow and tissues
www.pleasegiveblood.org/links/index.php
www.dexonline.com Find blood donor centers
www.BloodBankers.com (Free Nation wide directory)
www.bloodsafety.org

A new approach to improving how Red Cross collects and processes Blood; Quality Management system-training and Performance-operating systems-operating process-documentation system-equipment and facility-computer system.

American Society of Apheresis

www.apheresis.org
Dedicated to meeting the needs of apheresis practitioners, for training, continuing educations, development of resources for the donor recruitment profession.
Some Important Links Related to Blood Banking, Transfusion Medicine and Cellular and Molecular Therapies

American Society of Hematology
www.hematology.org

Blood Online
www.bloodjournal.org
International Society of Blood Transfusion (ISBT)
www.iccbba.com/
internationalsocietyofbloodtransfusionlong.htm
Transfusion Journals.

World Apheresis Association
www.worldapheresis.org
Umbrella organization, for National and International Professional Societies.

International Cord Blood Society
www.cordblood.org/public
Dedicated to the advancement of stem cell research with the emphasis on cord blood stem cells.

Plasma Protein Therapeutics Association
www.pptaglobal.org
Leading producers of plasma-based and recombinant biological therapeutics.

Center for Disease Control and Prevention (CDCP)
www.cdc.gov/nchstp/od/gap/strategies/2_3_blood_safety.htm
Excellent site for information to develop blood safety initiatives. Developed under the Global Aids Program. It provides much needed information for developing blood safety projects.

Canadian Blood Services
www.bloodservices.ca
See links

Canadian Standards Association
Health Canada: Guidance documents pertaining to blood
Health Canada: Good manufacturing practices guidelines.

FAQs Safety and Security
Frequently asked questions about blood safety and security of Canadian Blood System.

United Kingdom
National Blood Services Hospitals
www.blood.co.uk/hospitals/library/shot00d.htm
www.dh.gov.uk/policyAndGuidance/HealthAndSocialCareTopics/BloodSafety/Blood..

The National Blood Transfusion Committee. Provides national support and advice on national better blood transfusion initiatives.

South African National Blood Services
www.sanbs.org.za/
Excellent site for information on blood banking, donor recruitment and safe transfusion

WP Blood Transfusion Services
www.wpblood.org.za/

European Community
www.pei.de/english/safety.htm

World Health Organization
www.who.int/bloodsafety/gcbs/en/
Global Collaboration for Blood Safety (GCBS). This is a WHO convened forum, which is a voluntary partnership of internationally recognized organizations, institutions, associations, agencies and experts from developing and developed countries that are concerned with the safety of blood and blood products. The GCBS was established in response to the declaration of the Paris AIDS summit in 1994.

WHO Global Database on Blood Safety
www.int/bloodsafety/global_database/en/
The WHO Global Database on Blood Safety (GDBS) was established in 1997. Has been an invaluable tool for collection and analysis of data on blood transfusion services in all Member Sates of the World Health Organization.
Distance Learning Programs (WHO)
www.who.int/bloodsafety/education_training/en/
- Distance learning in blood safety
- National blood transfusion services
- Voluntary blood donation
- Testing and processing
- Safe appropriate use
- Quality management programme
- Education and training
- Global collaboration for blood safety
- Global database on blood safety

World Information about Blood Banking
www.eBloodBank.com
Site maintained by Chiron Blood Testing.

US Department of Health and Human Services
www.hhs.gov/bloodsafety/summaries/sumapr01.htm
Summaries of Executive Secretary, Advisory Committee on Blood Safety and Availability

US National Institutes of Health
www.nih.gov
Excellent site for information on stem cell research.

International Society for Cellular Therapy
www.celltherapy.org
Information on cord blood, islet cells gene therapy, dendritic cells, stem cell biology etc

Stem Cell Research Foundation
www.temcellresearchfoundation.org

Embryonic Stem Cell Research at University of Wisconsin
www.news.wisc.edu/packages/stemcells/

Stem Cell Therapy Information
www.cellmedicine.com

BMT/Stem Cell Therapy /Cord Blood
www.bloodline.net/bone-tem-cord/index

Bio Mark International
http://biomark/intl/com
Latest in targeted cell therapy and research.

Institute for Regenerative Medicine (IRM)
www.regenmed.com
Cellular therapy and tissue engineering.

Tropical Disease Institute Ohio
www.outcome.ohiou.edu/ARC/RedCross_TDI.htm
The Tropical Disease Institute at Ohio University is working with the American Red Cross in Central Ohio to improve blood safety in Ecuador.

Cryobanks International Cord Blood Donation and Storage
www.cryo-intl.com/samba.html
The Institute of Transfusion Medicine, Pittsburgh, USA
www.itxm.org/
Provides monthly transfusion medicine updates on transfusion-related issues

BloodMed.com
www.bloodmed.com/home/profile/asp
The global source for hematology education, practice and research.

Medical Academic Web Site
www.akademisyem.com/linkler/hematl.asp
15,000 journals, congresses, Powerpoint..

Network for Advancement of Transfusion Alternatives
www.nataonline.com

Blood Transfusion Tutorials and Case Studies
www.hoslink.com/bttut.htm

ATMRF-Advanced Transfusion Medicine Research Foundation
www.atmrf.org.in/

Baxter Transfusion Therapies
www.baxtertransfusiontherapies.com/tt/
Industry Information

www.octapharma.com
Makers of Octaplas-solvent detergent treated plasma for pathogen inactivation.

www.procrit.com
Suppliers of Epoietin Alfa for chemotherapy-induced anemia.

Software for Blood Bank and Transfusion Centers

www.mak-system.net

India’s Premier Medical Port

www.indmedica.com/drjolly.cfm
DR. JG Jolly former professor of Blood Transfusion Department of PGI Chandigarh is the consultant for this site maintained by Indmedica.

www.bloodsafety.com
Information provided by Pall Corporation: Overview of blood transfusion. Lists countries that have adapted Leukocyte filtration.

http://indiafocus.indiainfo.com/health/blood
Information on blood bank related topics in India.

Indian Society of Blood Transfusion and Hematology
Publishes journals and organizes annual meetings.

www.google.com

Rotary Blood Banks

Rotary Blood Banks, Dharawad, New Delhi, Bangalore, Chennai, Vishakapatnam, Mumbai, Kerala, Ahmedabad, Bhubaneshwar, Chandigarh, Bali (Indonesia), Nepal, Nigeria, St. Lucia, Cyprus.

Good Web Site of New Delhi Rotary Club

www.rotarybloodbank.org/home.htm
Provides useful information, gives credit to Rotary International. The Rotary route to Safe Blood

List of All Blood Banks in Karnataka

www.raktadan/bloodbanks.asp

List of All Blood Banks in Tamil Nadu
http://tnsacs.tn.nic.in/bloodbank.htm

List of All Blood Banks in New Delhi
http://dsacs.delhigovt.nic.in/blood.html
Overview

- Case
- Differential diagnosis
- Epidemiology
- Pathophysiology
- Lumpters and Splitters (TTP-HUS)
- Treatment

Case Report: EG

65-year old man with multiple episodes of TTP
- 1986
- 1991
- 1994
- 2001
Case Report: EG

- Just before 4 July, he was admitted for abdominal pain x 4 days, probable relapse of pancreatic pseudocyst.
- Over several days, platelet count drop from 129 ->73. WBC and coagulation tests remained normal.

Case Report: EG

- EG underwent plasmapheresis for suspected relapse of TTP
- Counts rapidly returned to normal
- Treatment complicated by Staph bacteremia
**EG Blood Counts**

- Compare time course for platelet count (left) and hemoglobin (right)
- Abrupt rise in platelet count follows initiation of plasmapheresis during each exacerbation of TTP
**Thrombotic Thrombocytopenic Purpura Classic Pentad**

- Thrombocytopenia
- Microangiopathic hemolytic anemia (MAHA)
- Hematocrit <30 percent
- Hemoglobin < 10 mg/dl
- LDH > 460 IU/L
- Haptoglobin – undetectable
- RBC fragmentation on peripheral smear

**Thrombotic Thrombocytopenic Purpura Classic Pentad**

- Thrombocytopenia
- MAHA
- Neurologic problem
- Coma
- Focal or generalized seizures
- Dysphasia
- Paresis
- Visual disturbances

**Thrombotic Thrombocytopenic Purpura Classic Pentad – Dire Outcome**

- Thrombocytopenia
- MAHA
- Neurologic problem
- Renal failure
- Fever

*Moschowitz 1924*

### Laboratory Findings in TTP (Fig 5.1)

<table>
<thead>
<tr>
<th></th>
<th>Increased</th>
<th>Decreased</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH RDW</td>
<td>Platelets HGB</td>
<td>WBC Coags</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 5.1**

**Thrombotic Thrombocytopenic Purpura Classic Pentad**

- Thrombocytopenia
- Platelet count < 150,000
Thrombotic Thrombocytopenic Purpura
Diagnostic Dyad (2000)

Primary Criteria
- Thrombocytopenia
- MAHA
- No obvious alternative

Other features
- Renal disease
- Neurologic abnormalities
- Weakness
- Abdominal symptoms
- Fever

UCSF Series of Patients

TTP in Pregnancy
Rakiva Kelly
- Pregnancy can trigger
- Differential:
  — HELLP (hemolysis, elevated liver enzyme levels and low platelet count) syndrome
  — DIC
  — ITP
  — Pre-eclampsia

Other TTP Associations
Female > Male (2:1)
Median Age in 40’s
Associations and Variants
- Bone marrow transplantation
- Autoimmune disease: SLE
- Infections: E coli, HIV
- Drugs: Ticlopidine, Clopidigril, mitomycin
- Malignancies
- Congenital: 9q32 ADAMTS gene
Arranging Cooked Pasta*

See Figure 5.2.
TTP vs DIC

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<thead>
<tr>
<th>TTP</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>MAHA</td>
<td>Rarely, fragments</td>
</tr>
<tr>
<td>Normal coagulation</td>
<td>Consumptive coagulopathy</td>
</tr>
<tr>
<td>Abnormal vWF</td>
<td>Endothelial and monocyte</td>
</tr>
<tr>
<td></td>
<td>tissue factor</td>
</tr>
<tr>
<td>Trigger may be inapparent</td>
<td>Triggers: sepsis or tissue</td>
</tr>
<tr>
<td></td>
<td>necrosis (tumor, abortion, etc.)</td>
</tr>
</tbody>
</table>

**Renal Arteriole Occluded by Thrombus in TTP** *(Fig. 5.8)*
- Platelet microthrombi cause vascular occlusion and
tissue hypoxia, particularly in the brain, kidneys, heart, lungs and spleen
- Red cells are mechanically destroyed
- Thrombi are rich in vWF and platelets, but not fibrinogen or fibrin.

**Von Willebrand Factor** (Fig 5.9)
- Critical megamolecule that initiates platelet adhesion and activation
- Produced in endothelial cells
- Stored in Weibel-Palade bodies
- Carries Factor VIII (hence, “Factor VIII-associated Antigen” [VIIIIRAg] in old literature)

**vWF Gene Product**
See Figure 5.10.
**vWF Multimers** (Fig. 5.11)

- vWF monomers (280KD) polymerize to form enormous (20MD+) multimers
- The larger the multimer, the more adhesive for platelets
- Proteolysis degrades multimers as they leave EC (Weibel-Palade bodies) or platelets (alpha granules)

**ADAMTS13** (Fig. 5.12)

- Metalloproteinase (Zn, Ca dependent)
- Disintegrin [arginine-glycine-aspartate (RGD) sequence]
- Thrombospondin1-like domain
- Binds to endothelial thrombospondin receptors, cleaving vWF as it is released

**Hypothesis**

Congenital or autoimmune dysfunction of ADAMTS13 prevents the normal proteolysis of large VWF multimers as they are secreted from injured endothelial cells.

Circulating UL VWF multimers are capable of supporting platelet aggregation more efficiently than normal multimers (especially at high shear)

**ADAMTS13 in TTP**

See Figure 5.13.
Platelet Adhesion to Ultra Large (UL) vWF

Labeled normal platelets in high shear system over histamine-stimulated endothelial cells
- Panel A: in buffer (no UL vWF cleavage)
- Panel B: in normal plasma (rapid UL vWF cleavage)
- Panel C: in TTP plasma (no UL vWF cleavage)

ADAMTS Defects

<table>
<thead>
<tr>
<th>Defect</th>
<th>Clinical syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMTS13 mutation (plasma activity &lt;5%)</td>
<td>Familial or chronic relapsing TTP</td>
</tr>
<tr>
<td>Autoantibodies to ADAMTS13 (transient,</td>
<td>Acquired idiopathic TTP</td>
</tr>
<tr>
<td>recurrent, or drug-induced)</td>
<td></td>
</tr>
<tr>
<td>Transient ADAMTS13 production or survival</td>
<td>Acquired idiopathic TTP</td>
</tr>
<tr>
<td>defect (theoretical)</td>
<td></td>
</tr>
<tr>
<td>Impaired ADAMTS13 attachment to endothelium,</td>
<td>Familial or acquired TTP</td>
</tr>
<tr>
<td>normal plasma activity</td>
<td></td>
</tr>
</tbody>
</table>

Recurrence or Familial TTP

<table>
<thead>
<tr>
<th>Recurrent non-familial</th>
<th>Familial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeated episodes</td>
<td>Repeated episodes</td>
</tr>
<tr>
<td>One family member</td>
<td>Multiple family members</td>
</tr>
<tr>
<td>TTP or HUS</td>
<td>TTP or HUS</td>
</tr>
<tr>
<td>Death or CRF or</td>
<td>Death or CRF or</td>
</tr>
<tr>
<td>Neurologic deficit</td>
<td>Neurologic deficit</td>
</tr>
</tbody>
</table>
Lumpers vs Splitters

See Figure 5.14.

Thrombotic Microangiopathies

<table>
<thead>
<tr>
<th>Type</th>
<th>Cause</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic platelet thrombi</td>
<td>Unusually large vWF multimers</td>
<td>TTP</td>
</tr>
<tr>
<td>Renal or systemic thrombi</td>
<td>Transplantation or drugs (mitomycin, cyclosporine, tacrolimus, quinine)</td>
<td>TTP-HUS</td>
</tr>
<tr>
<td>(Predominantly) renal platelet-fibrin thrombi</td>
<td>Exposure to Shiga toxin</td>
<td>Classic, childhood or E coli-associated HUS</td>
</tr>
<tr>
<td>(Predominantly) renal platelet-fibrin thrombi</td>
<td>Defect in plasma factor H</td>
<td>Familial or recurrent HUS</td>
</tr>
</tbody>
</table>

TTP-HUS

<table>
<thead>
<tr>
<th>TTP</th>
<th>HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Children</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Renal</td>
</tr>
<tr>
<td>Sporadic or drugs</td>
<td>Sporadic or E coli 0157:H7</td>
</tr>
<tr>
<td>Quinine, ticlopidine, mitomycin C, cyclosporine, pentostatin, gemcitabine</td>
<td>MAHA</td>
</tr>
<tr>
<td>MAHA</td>
<td>Thrombocytopenia</td>
</tr>
</tbody>
</table>

ADAMTS13 Activity

ADAMTS13 activity is low during acute episodes in patients with either recurrent/non-familial or familial forms:
- Panel A – TTP
- Panel B – HUS

Protease Level and Multimer Pattern

- High MW multimers occur even with normal protease
- Higher MW multimers present in remission.
TTP-HUS Paradigm

- Low values of ADAMTS13 activity aren’t specific for TTP, at least in recurrent and familial forms.
- Instead of the terms TTP and HUS, it is more appropriate to classify patients with thrombotic microangiopathies on the basis of the underlying specific defect; patients with ADAMTS13 deficiency are a subset.
- Deficient ADAMTS13 activity is not the only determinant of the presence of ultralarge VWF multimers in some phases of the clinical disease of patients with TTP-HUS.

Shiga Toxin Cascade (Fig. 5.15)

- Toxin binds to receptor
- Stimulates epithelial release of IL8 and chemokines
- Stimulates monocyte and epithelial release of TNFα, IL1, IL6
- Stimulates EC release of UL vWF
- Internalized toxin triggers apoptosis

Shiga Toxin in HUS

See Figure 5.16.

HUS Triggers

- Often follows prodromal infectious disease, especially diarrhea (90%) or URI (10%)
- Pathogens include *E. coli* 0157:H7, *Shigella, Salmonella, Yersinia, Campylobacter*
  — Verotoxin producers injure Vero [monkey] cells
  — Antimotility drugs may increase risk of HUS
- Other triggers: viral infections (varicella, echovirus, coxsackie A and B), *Strep pneumoniae, HIV, cancer and chemotherapy.*

Treatment of Thrombotic Microangiopathy

- Plasma exchange is better than plasma infusion.
- Pheresis should use FFP or cryosupernatant (depleted of vWF, fibrinogen and Factor XIII).
- If a delay is unavoidable, initiate therapy with plasma infusion.
- Continue pheresis daily until platelets, LDH, blood smear and neurologic status are back to normal, then taper over 1 – 2 weeks.
Plasmapheresis

- Draws off the EVIL humors (AKA UL vWF)
- Restores the GOOD humors (AKA ADAMST13)
- Dose: 60-80 ml/kg/d, median # of exchanges 9

Plasmapheresis Complications

- Bacteremia
- Catheter problems
- Allergic reactions
- Hypotension
- Citrate toxicity (paresthesias, tetany)

Other Therapeutic Options

- Glucocorticoids (10% response in pre-pheresis era)
- Uncertain value: dipyridamole, aspirin, azathioprine, vincristine
- Refractory: increase to BID exchanges, add prednisone, cryosupernatant, splenectomy?

Avoid Platelet Transfusions

- UCSF Series (n=55) of TTP patients, mortality was 7 times greater in patients receiving platelet transfusion.

Thrombotic Thrombocytopenic Purpura

Easy to diagnose and treat - if you think of it.

The five clinical features

- Thrombocytopenia
- Red cell fragmentation
- Fever
- Transient neurologic deficits
- Kidney failure

Untreated, TTP is deadly. Treatment usually involves replacing the plasma repeatedly until the patient recovers.

The usual problem, loss of a protein that removes activated VIII-R, is just now being figured out.

RBC Fragments

Essential anatomic lesion: widespread thrombin-platelet microthrombin.
Platelet Adhesion Receptor (Fig. 5.17)

- Glycoprotein Ib/IX/V complex
- Cytoplasmic tail interacts with platelet cytoskeleton
- When receptor is occupied, platelet is activated
- Main occupant: vWF

Platelet Aggregation Receptor (Fig. 5.18)

- Glycoprotein IIb/IIIa complex
- Platelet activation required for receptor to bind fibrinogen
- Main occupant: Fibrinogen (but vWF can also occupy)
vWF Domains and Mutants

See Figure 5.20.

### Priorities
- Definition – develop explicit criteria
- Diagnosis
- Treatment
- Prognosis

### Table 5.1: Thrombotic microangiopathies

<table>
<thead>
<tr>
<th>Type of microangiopathy</th>
<th>Cause</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic platelet thrombi</td>
<td>Failure to degrade unusually large multimers of von Willebrand factor</td>
<td>Thrombotic thrombocytopenic purpura</td>
</tr>
<tr>
<td>Predominantly renal platelet-fibrin thrombi</td>
<td>Exposure to Shiga toxin</td>
<td>Classic, childhood, or <em>Escherichia coli-</em> associated hemolytic - uremic syndrome</td>
</tr>
<tr>
<td>Renal or systemic thrombi</td>
<td>Defect in plasma factor H, Transplantation or drugs (mitomycin, cyclosporine, tacrolimus, quinine)</td>
<td>Familial (or recurrent) hemolytic-uremic syndrome, Hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura</td>
</tr>
</tbody>
</table>

### Table 5.2: Relation between defects in plasma von Willebrand factor-cleaving metalloprotease, ADAMTS 13, and thrombotic thrombocytopenic purpura (TTP)

<table>
<thead>
<tr>
<th>Defect</th>
<th>Clinical Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMTS 13 plasma activity &lt; 5% of normal</td>
<td>Familial TTP, chronic relapsing TTP</td>
</tr>
<tr>
<td>Mutations in the gene for ADAMTS 13</td>
<td></td>
</tr>
<tr>
<td>Disease presentation in infancy or childhood</td>
<td></td>
</tr>
</tbody>
</table>

Contd...
### Defect Clinical Presentation

<table>
<thead>
<tr>
<th>Defect</th>
<th>Clinical Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease presentation later in life</td>
<td>Acquired idiopathic TTP</td>
</tr>
<tr>
<td>Autoantibodies against ADAMTS 13</td>
<td>Single-episode TTP</td>
</tr>
<tr>
<td>Transient</td>
<td>Recurrent (intermittent) TTP</td>
</tr>
<tr>
<td>Recurrent</td>
<td>Ticlopidine-associated TTP</td>
</tr>
<tr>
<td>Ticlopidine-associated*</td>
<td>Acquired idiopathic TTP</td>
</tr>
<tr>
<td>Transient defect in production or survival of ADAMTS 13†</td>
<td>Familial and acquired TTP</td>
</tr>
<tr>
<td>Normal ADAMTS 13 activity in plasma with defective attachment of ADAMTS 13†</td>
<td></td>
</tr>
</tbody>
</table>

* Cases associated with clopidogrel, which is structurally similar to ticlopidine, have been reported.
† This possibility has yet to be proved.

**EG MCV**

![MCV Graph]

**EG Hgb**

![Hgb Graph]