CONTENTS

79
Letter to the Readers
Introduction to the review articles
S. Murphy, MD

Review: platelet matching for alloimmunized patients—room for improvement
S. T. Nance, S. Hsu, R. R. Vassallo, and S. Murphy

89
Review: platelet alloantigens and antibodies and their clinical significance
A. Norton, D. L. Allen, and M. F. Murphy

103
Review: transfusion-related acute lung injury: pathophysiology, laboratory investigation, and donor management
P. M. Kopko

112
Review: immune thrombocytopenic purpura: an update for immunohematologists
S. G. Sandler

118
The sensitivity, specificity, and clinical relevance of gel versus tube DATs in the clinical immunology laboratory
N. Paz, D. Itzhaky, and M. H. Ellis

122
COMMUNICATIONS
Letter to the Editors
An unusual antibody reacting with pre-diluted 0.8% reagent RBCs and with 0.8% older (aged) RBCs prepared at the time of testing
J. Trimble

125
Letters From the Editor-in-Chief
The second 20th anniversary issue!
My sincere apology
Memories from the past! What is next?

126
SPECIAL SECTION
Excerpts from the Red Cell Free Press

128
Book Review
Stem Cells Handbook
N. Rebecca Haley, MD

131
CLASSIFIED AD

134
INSTRUCTIONS FOR AUTHORS
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Introduction to the review articles

As part of the celebration of the 20th anniversary of Immunohematology, this issue contains four review articles that take the reader from the serology laboratory to the bedside and back. This constant interplay between the laboratory and the patient makes immunohematology an extraordinarily rewarding endeavor. The first three articles describe clinical mischief caused by antibodies to human platelet antigens (HPA), human leukocyte antigens (HLA), human granulocyte antigens (HGA), and human monocyte antigens. The fourth review describes a red blood cell antibody that went from the bedside to the laboratory and then returned to the bedside as therapy for patients with low platelet counts.

S. Nance et al. describe the exploits of a young physician, Dr. Peter Petz-Garratty, and then compare and contrast the evolutions of red blood cell matching and platelet matching. Most patients with refractoriness to platelet transfusion have HLA antibodies. The authors conclude that perhaps methods of platelet matching should be modeled after red blood cell matching. In fact, this possibility has been made more attractive by the development of new, more sensitive methods to determine the specificities of HLA antibodies.

A. Norton et al. provide a thorough review of the HPAs that are present on platelets but not on other blood cells. During pregnancy, a woman may develop HPA antibodies, and they, in turn, may cross the placenta to produce thrombocytopenia in the fetus (NAIT). In other patients, HPA antibody may be produced in response to blood transfusion associated with the puzzling syndrome posttransfusion purpura (PTP).

P. Kopko then grapples with the syndrome of transfusion-related acute lung injury (TRALI). This may now be the second most common cause of death, after transfusion, and there can be considerable morbidity for those who survive. The etiology is hotly debated. Most, but not all, cases develop after a transfusion containing a relatively large volume of plasma, as in fresh-frozen plasma or apheresis platelets. Antibodies to leukocytes and monocytes are often found in the donor’s plasma. In a typical case, the antigen to which the antibody has formed is present on the patient’s white blood cells.

Finally, G. Sandler discusses the clinical characteristics and pathophysiology of idiopathic thrombocytopenic purpura (ITP). Recently, several therapeutic modalities have been added to the old standbys, corticosteroids and splenectomy. These include intravenous gamma globulin (IVIg), WinRho (sometimes referred to as anti-D), and Rituximab. For half a century, the ability to detect anti-D, using the antiglobulin test, has been crucial to the management of many patients. Now it has returned to the bedside as a therapeutic agent.

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Review: platelet matching for alloimmunized patients—room for improvement

S.T. NANCE, S. HSU, R.R. VASSALLO, AND S. MURPHY

A Parable

A 40-year-old woman was admitted at midnight to HMO General Hospital for evaluation of profound fatigue. Her hemoglobin was found to be 2 g/dL. Her medical history included several pregnancies and transfusions. The intern had attended a lecture on transfusion medicine that day. He ordered a “six-pack,” six units of random-donor RBCs. The technologist on call for the blood bank was a bacteriologist, cross-covering the transfusion service. The technologist removed six units of RBCs from the refrigerator, pooled them, and sent a liter of the mixture to the floor for transfusion, which was quickly followed by back pain and black urine for the patient.

The intern called the on-call transfusion service medical director, who was cross-covering from cardiology. The intern asked, “Can’t you do immunologic testing or provide a matched product?”

The director responded, “No way! What was the 1-hour CCI? We insist upon two documented failures before we do an immunology workup. I’m sending the patient another pool.”

“Oh no, not again!” said the patient. Shortly after transfusion, back pain and black urine recurred.

The resident called the medical director and said, “That’s two, Doc!”

“OK. We’ll phenotype the patient’s RBCs and search for a good BX match in inventory. You know, Jk⁺ and Jk⁻ are crossreactive.”

Once again the patient said, “Are you sure?” However, the transfusion was given and soon she was having back pain and black urine again.

In desperation, the medical director said, “I’ll take random units off the shelf and crossmatch until I find a compatible unit.” The unit was found and transfused and there was no back pain or black urine until 5 days later.

The next day, a new resident, Dr. Peter Petz-Garratty, came on service. He reviewed the chart and said, “Why don’t you examine the patient’s serum for antibodies and determine their antigenic specificities? Then, crossmatch products without those antigens.”

The medical director said, “This man has a future.”

Evolution of Strategies for RBC Matching

Practice was quite primitive when physicians first considered transfusing blood from one individual to another. They began transfusing whole blood between experimental animals, without any consideration whatsoever for RBC antigen-antibody compatibility. Interestingly, this lack of compatibility testing persists even today for the majority of platelet transfusions.

In an excellent historical review, Myhre described a report by John Wilkins of a dog-to-dog transfusion of about 2 ounces of blood via syringe. At that time, it was not recognized that there was a possibility of naturally occurring alloantibodies (not present in dogs) or the relevance of a volume possibly too small to produce a reaction. Other investigators in London and France also experimented with animal-to-animal transfusions. This was done, of course, without compatibility testing.

Since animal-to-animal transfusions went quite well, soon the time came to try animal-to-human transfusions. Jean Baptiste Denys performed the first animal-to-human transfusion in mid-1667. Nine ounces of lamb’s blood was transfused to a 16-year-old patient who had been bled at least 20 times over 2 months. During the transfusion, it was reported that the patient experienced “very great heat along his arm.” Again, no testing of the blood or the recipient was performed.

James Blundell was a major contributor to the early years of human-to-human blood transfusion. He reported a transfusion to a patient with obstinate vomiting, in 1819. The donors were several physicians;
12 to 24 ounces of blood was given by syringe.² This is reminiscent of the use of six packs for platelet transfusion today. As in the preceding two accounts, no laboratory testing was carried out on the donor or recipient blood. These early reports share relatively small transfused volumes and a lack of pretesting. One report remarked that animal blood was thought to be more pure, as the animal did not participate in some of the risky pursuits that humans did.

Fast forward to today, when involved parties’ blood, both donor and recipient, are subjected to a large battery of tests. But how did the testing algorithm develop, and why? As mentioned previously, the first transfusions were given without testing of the donor or the recipient. Interest in transfusion waned in the late 19th century due to a high rate of severe adverse reactions to transfusion.³ Landsteiner's experiments and direct transfusions by Carrel, described by Crile,⁴ promoted a recurrence of interest. The subsequent determination of ABO groups gave rise to renewed interest in human-to-human blood transfusion.

In a publication by Ludwig Hektoen,⁵ what would become the major and minor crossmatches were described in 1907. In the early days, the methods concentrated solely upon detection of room temperature IgM antibodies, as the “Coombs test” had not yet been described. Thus, these investigators were studying ABO compatibility. Ottenberg⁶ and Epstein⁷ studied a method for hemolysis and agglutination tests. Epstein promoted a pretransfusion crossmatch, using tests both for hemolysis and for agglutination. It was later recognized that when agglutination was present hemolysis might also be present, but in vitro hemolysis did not occur without agglutination.⁸ With this information, agglutination tests could be used without tests for hemolysis. At this time, all testing still concentrated upon detection of IgM antibodies. Changes like the recommendation for testing at 37°C and concentration upon the major crossmatch followed. During World War I, Lee proposed that if type O blood were used, preliminary testing could be avoided.⁹

These red cell innovations in the early part of the 20th century were quite similar to the use of “blind” crossmatching to assess platelet compatibility today. The first practical method for platelet crossmatching was not described until 1988.¹⁰ This will be discussed further in the next portion of this paper.

Major changes to detect IgG antibodies followed the reports of enhancement of agglutination with bovine albumin¹¹ and antiglobulin testing.¹² Although other enhancement media and techniques (enzyme, LISS, PEG, and solid phase and column agglutination technology) have been described, the basic premise of the major crossmatch remains remarkably the same, i.e., with testing of the patient’s serum against donor RBCs.

At first, the minor crossmatch was also felt to be necessary, but a paradigm shift occurred. The minor crossmatch was eventually abandoned in favor of routine donor antibody screening. The need for complement-reactive antihuman globulin (AHG) sera has been eliminated because of the rare occurrence of clinically significant antibodies leaving only complement on the cell surface. The requirement for serum as a source of a test sample has been changed for the same reason.

Another change was the departure from room temperature testing to 37°C for detection of potentially significant antibodies. Most clinically significant antibodies are reactive at 37°C, and antibodies reactive solely at room temperature are generally not thought to have clinical significance.

More recently, AABB standards have allowed the use of immediate spin testing as long as the patient’s antibody screen is negative. In some laboratories, electronic crossmatches have taken the place of the AHG crossmatch. Thus, few patients’ samples are routinely subjected to an AHG crossmatch. When the antibody screen is positive, RBC units are selected that lack antigens to which the antibody is directed. This is quite similar to the evolution of antibody-based matching for platelets.

The number of RBC transfusions requiring a full crossmatch is proportionate to the number of platelet transfusions currently guided by crossmatch testing, but for vastly different reasons. In the case of potential RBC recipients, an AHG crossmatch will be required by the serologic determination of alloantibodies or autoantibodies. In the case of potential platelet recipients, however, crossmatching is ordered after several transfusions have not yielded expected increments. The present status of platelet crossmatching, then, parallels the state of RBC testing in the early 1900s.

**Evaluation of Strategies for Platelet Matching**

In current practice, platelet transfusion begins with randomly selected platelet products, either pooled whole-blood–derived platelets or platelets prepared by apheresis. This is analogous to the initial management
of the anemic woman in the parable. This approach continues until the patient is refractory, i.e., fails to show adequate increments after transfusion. To identify a patient as refractory, most clinicians require two or three failed transfusions. Again, this was the way the patient in the parable was managed. Commonly, increments are expressed as corrected count increments (CCI) where

\[
CCI = \frac{\text{Plt. Ct. Increment (}/\mu\text{L)} \times \text{Body Surface Area (m}^2\text{)}}{\text{Plts. Transfused (} \times 10^{11} \text{)}}
\]

Refractoriness is commonly defined as a CCI less than 4000 per µL per m² per 10¹¹ platelets. Therefore, Duquesnoy et al. defined matches between donor and patient as A, HLA identical; BU, (partially homozygous) donor antigens all present in the recipient; BX, all donor HLA antigens either identical to or within recipient CREGs; C & D, one or more donor antigens not present in recipient or in recipient CREGs. This is analogous to the phenotypic matching of RBCs.

The use of BX matches greatly expanded the number of potential donors. This was an advance because many BX matches provided good increments in the refractory patient. However, the predictive power of this approach was poor. Poor responses were frequent with BX matches and a surprising number of good responses were seen with C and D matches. The patient in the parable had a poor response to a “phenotypic match” at the Kidd locus.

The classical HLA approach to platelet matching began with the paper by Yankee et al. that compared the use of platelets from HLA-identical siblings with randomly selected platelets in refractory patients. There were no increments with random products but excellent responses to the perfectly HLA-matched sibling donors.

Most immunologic platelet refractoriness results from antibodies to HLA. It is important to emphasize that clinical refractoriness is often due not to alloimmunization but, rather, to clinical factors such as infection, splenomegaly, or disseminated intravascular coagulation. Thus, it would make sense to perform one or more serologic tests to prove that alloimmunization was present. However, this approach was rare during the first 20 to 25 years of matched-platelet transfusions.

The classical HLA approach to platelet matching began with the paper by Yankee et al. that compared the use of platelets from HLA-identical siblings with randomly selected platelets in refractory patients. There were no increments with random products but excellent responses to the perfectly HLA-matched sibling donors.

It is generally not realistic to support refractory patients with sibling donors. Duquesnoy et al. introduced the concept of supporting patients with HLA-matched platelets from the general population. However, they noted that a pool of 50,000 donors would be required to provide a 50 percent probability of finding ten perfectly matched donors for each of 100 potential recipients. This was also unrealistic. They proposed a method that we will call the CREG method. Cross-reactive groups (CREGs) of HLA antigens have been defined by serologic testing. Cross-reactivity among antigens within a CREG results from the sharing of one or more public epitopes. Patients do not make antibody to their own HLA antigens nor commonly to antigens within CREGs of their own HLA antigens. Therefore, Duquesnoy et al. defined matches between donor and patient as A, HLA identical; BU, (partially homozygous) donor antigens all present in the recipient; BX, all donor HLA antigens either identical to or within recipient CREGs; C & D, one or more donor antigens not present in recipient or in recipient CREGs. This is analogous to the phenotypic matching of RBCs.

In our laboratory, one half of patients referred for matched platelets have no antibody. We discourage matching in such patients except in the rare circumstance that immunologic refractoriness is mediated solely by antibodies to human platelet antigens (HPAs). The results of the AHG-CDC assay determine not only the presence or absence of antibody, but also the breadth of immunization expressed as the percent reactive antibody (PRA), the percentage of wells in which lymphocytotoxicity is...
seen. Furthermore, by analyzing the positive wells, one frequently can determine the specificities of the antibodies. When we analyze results with the LCT assay in refractory patients, the PRAs can range from 1 percent to 100 percent, so one can classify patients as mildly (1–40% PRA), moderately (40–70% PRA), and severely (PRA > 70%) immunized.

Furthermore, when the specificities are determined, frequently one can identify intra-CREG antibodies: antibodies against antigens within the patient’s CREGs. With these facts in mind, one can understand why the CREG method has a relatively poor predictive capacity. When a BX match fails, there may be intra-CREG antibody. When a C or D match succeeds, the patient, although immunized, may have no antibody against the antigens present on the transfused platelets. There is a computer program available to better predict successful BX and C and D matches.22,23 It is known as “HLA Matchmaker.”

Furthermore, donor selection may be improved by “HLA Matchmaker.” It is based upon the concept that HLA molecules express their antigenic determinants as amino acid triplets. These triplets are shared among otherwise unrelated HLA molecules. Antigens whose triplet complement is identical to ones present in the patient are highly likely to provide compatible products for an immunized patient. BX matches with fewer triplet mismatches may be more likely to succeed, as would triplet-identical C and D matches. This concept awaits validation in a clinical study.

A quite different approach, crossmatching of donor platelets with a patient’s serum, was introduced in 1988 and, later, in 1997.10,24 The most commonly used method, at least in the United States, has been the solid phase red cell adherence (SPRCA) assay, which is available commercially as Capture-P from Immucor, Inc. (Norcross, GA). The platelets of the potential donor are layered in microtiter wells and the patient’s serum is added. The platelets are then washed and anti-IgG–coated indicator RBCs are added. If there is antibody to the platelets, the RBCs form a thin film in the well. If there is no antibody, the RBCs puddle in a button on the bottom of the well.

Many centers simply take platelets from inventory and perform “blind” crossmatching, with no pre-selection of the donors by identifying the antigens to which the patient has formed antibodies. This stands in contrast to RBC matching, in which cells are deliberately chosen for crossmatching that lack the antigens to which the patient has been sensitized.

The crossmatch method has the advantage of being fast, once the patient’s serum is in hand. In addition, it is a form of screening for the presence of antibody. In our experience21 comparing the SPRCA to the AHG-CDC assay, both tests detect antibody in 50 percent of referred patients, while all crossmatches are compatible in 30 percent. It is very rare for the SPRCA to be entirely negative (compatible) when the AHG-CDC is positive. Thus, if all crossmatches are compatible, it is very unlikely that the patient is refractory for immunologic reasons. On the other hand, 20 percent of these cases are SPRCA positive and AHG-CDC negative. The majority of such cases are seen in group O patients who have positive reactions to donors who are group A and/or B. In this situation, the positive crossmatch would be considered falsely positive since the platelets may be an excellent HLA match.

Recent results from Gelb and Leavitt24 in 76 potentially immunized patients indicated a mean CCI of 1800 for randomly selected products and 9800 for crossmatch-compatible products. However, there were no compatible products for ten patients. Another mode of support based on HLA matching might have helped these ten patients. Furthermore, in the other 66 patients, the mean percentage of screened units that were compatible was 69 percent (range: 24–100%), suggesting that these patients were only mildly or moderately immunized. Overall, 59 percent of crossmatch-compatible products produced CCIs greater than 7500. On the other hand, 41 percent did not.

Crossmatching is an excellent way to begin support when a patient needs a product urgently, if appropriate technical staff are available (i.e., perhaps not on nights or weekends). It provides a quick answer to the question of whether or not the patient is alloimmunized. In our experience, it is generally satisfactory for mild and moderate degrees of alloimmunization but less helpful for the severely immunized. Furthermore, it may find a very good HLA match incompatible because of ABO antibodies in the patient. In our experience21 with highly immunized patients, the majority of crossmatch-compatible products are not successful unless the degree of HLA compatibility is high.

A final approach was suggested by Dr. Peter Petz-Garratty in the parable. Why not examine the patient’s serum for antibodies and determine their antigenic specificities? Then, provide antigen-negative products, perhaps with a final check by crossmatching. In fact, this approach was evaluated by Petz and Garratty et al.25 in a large number of alloimmunized patients. They
called it the antibody specificity prediction (ASP) method. The specificities of the patient's antibodies were determined with the AHG-CDC method and antigen-negative platelets from an HLA-typed donor pool were selected for transfusion. This approach was compared to “blind” crossmatching. The increments were better with the ASP method, although not statistically so since there was a large range around the means for both methods. The authors also found that a perfect A or BU match produced the best result.

We studied the PRA of patients over time, up to a maximum of 16 months. Our results were similar to those of Lee and Schiffer. PRAs tend to remain constant over time, even as the patients continue to be transfused. On the other hand, approximately 25 percent of immunized patients may lose their antibodies over a period of months, a phenomenon that we do not understand. In practice, this means that one can perform an AHG-CDC assay once monthly to monitor the patient’s status without concern for major changes during the period.

A great advantage of the ASP method is that it increases the number of potential donors. Further, if one has identified the specificities of the patient's antibodies, one can distribute compatible products from inventory at any time without the need for testing. Our approach is outlined in Table 1.

It is clear that what Petz and Garratty et al. are suggesting brings us to a method very similar to what we do with RBCs. We are using more complex methods but their cost is less than that of a failed matched-platelet product. With current methods, frequent failed platelet transfusions are accepted routinely. We would not tolerate this with RBCs, since morbidity from an incompatible RBC transfusion can be severe. However, there is morbidity from a failed platelet transfusion: a continuation of the patient's bleeding risk.

In addition, a new generation of technologies developed and used for organ transplantation can also be used for the platelet-refractory patient. These are discussed in detail in the next section. Briefly, new techniques for determining the specificity of antibodies take advantage of the ability to use isolated, single HLA molecules in ELISA or flow cytometric-based assays. This is in contrast to the AHG-CDC assay, in which two to four antigens are expressed on each target lymphocyte.

In addition, there are simple ELISA methods in which the target is a wide variety of HLA antigens. One could use these methods to screen patients for the presence of HLA antibodies, perhaps every 2 to 4 weeks. With this approach, one can be proactive and begin matching earlier. We could abandon the practice of forcing patients to “earn” their matched products by experiencing several therapeutic failures.

**Platelet Matching: New Technology for the Future**

For solid organ transplantation, the detection of circulating anti-HLA antibodies in allograft recipients correlates not only with hyperacute, acute, accelerated, and chronic graft rejection, but also with reduced graft function and graft failure. Over the past 40 years, a plethora of papers has been published, discussing the merits of various antibody detection methodologies in relation to the clinical outcomes of solid organ transplants. In contrast, very little can be found in the literature relating these methods to platelet transfusion outcome. This may partly be due to the difficulty of such research due to the confounding effect of variables such as storage-related changes in platelet viability, concurrent recipient nonimmunologic platelet destruction, and the difficulties of demonstrating clinically significant changes in outcome in the context of prophylactic platelet use. This meager literature is further confused by the use of less sensitive “gold standard” assays (unenhanced lymphocytotoxicity tests) for antibody screening in many comparative studies.

The objective of this portion of the review is to discuss different testing methodologies currently in use for the detection of anti-HLA antibodies as they relate to platelet transfusion. We will also outline what we believe to be the optimal approach to characterizing
the HLA antibody profile of highly sensitized platelet-refractory patients.

HLA Class I antigens are expressed on all nucleated cells, including platelets. HLA Class I antibodies are relevant not only to solid organ and tissue transplantation, but also to WBC and platelet transfusion. Class II antigens are expressed on B cells, monocytes, macrophages, dendritic cells, and activated T cells. HLA Class II antibodies are therefore unimportant in the context of platelet transfusion.

Within the relevant Class I HLA-A and HLA-B families (HLA-C being present on the platelet surface in a density too low to be clinically important), there are several CREGs, which express multiple public epitopes. These public epitopes are common antigenic determinants shared by different HLA antigens. HLA-B locus antigens have two mutually exclusive public epitopes, Bw4 and Bw6, as well as a number of others defining the CREGs, such as 5C, 7C, 22C, 27C, 8C, 12C, and 21C. Private epitopes refer to antigenic determinants specific for an individual antigen. In addition to “foreign” CREGs, patients can occasionally make antibodies to antigens within their own CREGs, either to private epitopes or to different public epitopes within a CREG.31 The CREG antibody profile remains remarkably constant over time in patients.31,32 Monthly fluctuations in PRA, if not due to new transfusion events or changes in assay technique or panel composition, usually reflect the waxing and waning of detectable specificities within the same CREG, or they can reflect the increasing strength or number of reactivities within the same CREG cluster.30

In the transfusion setting, we have observed that greater than 50 percent of sera screened by flow cytometric methods contained both IgM and IgG antibodies.35 These are mostly directed against public epitopes or high-frequency private epitopes present in transfused units. Additionally, some antibodies arise from an anamnestic response from prior sensitization to paternal antigens during pregnancy.34,35

Antibody screening provides information regarding the presence or absence of HLA antibodies, the PRA against HLA alloantigens, antibody specificities, the immunoglobulin isotypes produced, and even antibody titers. This information may in turn be used to predict products likely to provide successful patient platelet concentration increments.21,25 The ideal screening method should be sensitive enough to detect the lowest level of clinically relevant IgM and/or IgG antibodies.

The original NIH two-stage complement-dependent cytotoxicity assay (CDC)36 used for HLA phenotyping is illustrated in Figure 1,39 which depicts the two steps of the basic CDC method with alternative steps (indicated by asterisks) included to illustrate (1) extended incubation to allow low avidity/affinity antibodies to bind cellular antigens; (2) a wash step to remove unbound serum and minimize “anticomplement factors,” which may inhibit or weaken complement activity (Amos modified technique)37; and (3) goat AHG-CDC, which overrides inefficient C1q binding by single IgG molecules.38,39 Many of the anti-public or “CREG” antibodies are not detectable by the standard CDC method due to inefficient complement activation by HLA antibodies. This is termed the cytotoxic-negative, adsorption-positive phenomenon (CYNAP).40 The AHG-CDC method is the most sensitive of the four complement-dependent cytotoxicity tests. In some laboratories, the sensitivity of the AHG-CDC is comparable to the multiple-antigen-panel ELISA-based technique.

The major advantage of complement-dependent assays is the ability to detect both IgG and IgM antibodies. While IgM anti-HLA antibodies do not play a role in solid organ transplantation, this may not be the case in platelet transfusions. The disadvantages of complement-dependent assays include the detection of not only HLA-specific antibodies but also nonspecific specificities or autoantibodies. The latter can be eliminated by heating the serum at 63°C for 10 minutes.
treat ing the serum with DTT, or performing an autorecrossmatch in parallel. CDC-determined PRAs may fluctuate due to changes in panel cell composition or poor viability of frozen cells. Without prior adsorption, sera obtained from patients receiving OKT3 or anti-T cell immunoglobulins cannot be tested by CDC methods.

There are two forms of solid phase–based methods: the ELISA41,42 and flow cytometric assays.33–45 These methods employ purified soluble HLA antigens affixed to solid phase matrices (plastic trays for ELISA or beads for flow cytometry) as targets for the binding of HLA antibodies instead of the live cells employed in CDC assays.

The advantages of solid phase–based methods are the ability to distinguish between IgG and IgM isotypes, better detection of HLA-specific antibodies, objective end-result determination by instrument optical density readings, the lack of assay interference by OKT3 treatment, and faster turnaround time.

Currently, ELISA assays are available in two or three formats. The first is the screening test for the presence or absence of HLA antibodies. A pool of HLA antigens is affixed to a single well for this purpose. Positive sera are further defined using a panel of HLA antigens purified from EBV cell lines or platelets to allow the determination of PRA and antibody specificity. The sensitivity and specificity of ELISA using heterozygous antigen panels (i.e., each well coated with multiple antigens) is comparable to that of the AHG-CDC method. A third type of ELISA tray is the high-definition (HD) or single-antigen tray. Each well in the tray is coated with a single HLA Class I–soluble antigen (HD) or single-antigen tray. Each well in the tray is coated with a single HLA Class I–soluble antigen derived from recombinant DNA technology.46 This high-definition antigen panel provides the ideal tool to characterize antibody specificities contained in high-PRA sera. Both the sensitivity and the specificity of screening sera using high-definition ELISA trays are increased when compared with the AHG-CDC method.47 In general, the ELISA method is not sensitive for the detection of IgM HLA antibodies.

Similar to the variety of ELISA methods, there are also three kinds of flow beads available: polyspecific beads, useful as a screening tool; beads coated with heterozygous soluble Class I antigens to determine PRA; and antibody specificity or high-definition flow beads. Currently the high definition beads consist of 34 individual recombinant A locus antigens, 57 B locus antigens, and 19 C locus antigens.

Recently, another flow cytometric platform was developed, based upon Luminex Microsphere (bead) technology. This technology combines two of the most powerful detection and identification tools, the exquisite binding specificity between biotin and streptavidin and the versatility of the flow cytometer. By the precise blending of different fluorescent intensities of the two dyes, 100 unique color codes are created. When the colored beads are coated with purified Class I antigens, they can be used for screening and identification of HLA antibody specificities. Although a number of large parallel studies between standard and Luminex flow methods are still in progress, data published thus far indicate that they are likely to be comparable to each other.47

It is well established that flow cytometry methods are more sensitive than AHG-CDC and ELISA. Better clinical correlations are obtained using flow methods not only for antibody screening but also for transplantation crossmatches. Flow methods can detect extremely low-level antibodies undetectable by either AHG-CDC or ELISA methods.46–50

Considering reported sensitivities and specificities together with our own comparison studies of AHG-CDC versus ELISA or flow methods,53 our ranking of preferred antibody elucidation methods for platelet-refractory patients is: flow cytometry (HD panel) > ELISA (HD panel) > AHG-CDC.

The optimal approach to characterizing the HLA antibody profiles of highly sensitized patients, in our opinion, is to HLA type potential platelet recipients and to perform a thorough determination of the antibody specificities using either ELISA or flow methods on high-definition antigen or bead panels. Knowledge of the recipient’s HLA type assists in analyzing the antibody profiles. Patients can only produce HLA antibodies against mismatched public and private epitopes. Patients are less likely to be sensitized by antigens belonging to their own CREG groups, although intra-REG antibody formation is not uncommon.

Screening high-PRA individuals using high-definition trays sometimes results in near-100 percent panel reactivities. This is mostly due to the presence of high-titer antibodies. We now use high initial optical density readings (O.D. > 2.0) from the screening assay for the presence of HLA antibodies as a guide to dilute these high-titer PRA sera 1:10 or 1:20 for subsequent screening on high-definition antigen or bead panels. The few antigens to which these individuals are not sensitized may be confirmed with compatible lymphocyte crossmatches from products negative for the corresponding antigens.
Our ultimate goal is to rapidly identify individuals with HLA Class I antibodies and attempt to provide so-called antigen-negative platelet products, even to highly sensitized patients, akin to the traditional approach outlined above for RBC transfusions. While this approach may be somewhat expensive compared with the current practice of therapeutic trial and error, the savings engendered by early identification of alloimmunization and consequent avoidance of failed transfusion, prolonged thrombocytopenia, and associated morbidity and costs may be justified in the long run.

References


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Antigens on human platelets are categorized according to their biochemical nature into carbohydrate antigens on glycolipids and glycoproteins (GPs) (A, B, O, P, Le antigens), protein antigens (human leukocyte antigens [HLA] Class-I A, B, and C, GPIIb/IIIa, GPIb/IX/V), and haptens (quinine, quinidine, heparin, and some antibiotics, e.g., penicillins and cephalosporins).

Many platelet antigens are shared with other blood cells, e.g., ABO and HLA class I antigens, but some of the glycoprotein antigens are expressed predominantly on platelets. These antigens are commonly referred to as platelet-specific alloantigens or human platelet alloantigens (HPAs), although some of these are also present to a lesser extent on other blood cells, e.g., HPA-5 on activated T lymphocytes.

### Human Platelet Alloantigens

There are a number of well-characterized biallelic platelet alloantigen systems, and a number of rare, private, or low-frequency antigens have also been described (Table 1). Most of these antigens were first discovered during the investigation of cases of neonatal alloimmune thrombocytopenia (NAIT).

Platelet-specific alloantigens are located on platelet membrane GPs involved in hemostasis through interactions with extracellular matrix proteins in the vascular endothelium and plasma coagulation proteins. The majority of these antigens are on the GPIIb/IIIa complex, which plays a central role in platelet aggregation as a receptor for fibrinogen, fibronectin, vitronectin, and von Willebrand factor. Other important GPs are GPIb/IX/V, the main receptor for von Willebrand factor involved in platelet adhesion to damaged vascular endothelium; GPIa/IIa, which is involved in adhesion to collagen; and CD109, which also appears to be a collagen receptor. Congenital deficiency of these GPs results in bleeding disorders, e.g., lack of GPIIb/IIIa causes Glanzmann's thrombasthenia, and absence of GPIb/IX/V results in Bernard-Soulier syndrome. The expression of platelet alloantigens located on these GPs may be altered in these disorders, and HPA typing performed by serologic assays (phenotyping) may give discrepant results when compared with results obtained by molecular typing (genotyping).

#### Inheritance and nomenclature

Most of the platelet-specific alloantigen systems reported to date have been shown to be biallelic, with each allele being codominant. Historically, systems were named by the authors first reporting the system, usually using an abbreviation of the name of the patient in whom the antibody was detected. Some systems were published simultaneously by different laboratories and with different names, e.g., Zw and Pl A, or Zav/Br/Hc, and only later were they found to be the same polymorphism. In 1990, a working party for platelet immunology of the ISBT agreed on a new nomenclature for platelet polymorphisms, the Human Platelet Antigen (HPA) nomenclature. Any new platelet GP alloantigens are now accepted and named according to guidelines established by the recently founded International Platelet Nomenclature Committee.¹

In the HPA nomenclature, each system is numbered consecutively (HPA-1, -2, -3, and so on) according to its date of discovery. The high-frequency allele in each system is designated “a” and the low-frequency allele “b.” Newly discovered systems are only officially included when confirmed by a second party and approved by the nomenclature committee. If an antibody against only one allele has been reported, a “w” (for workshop) is added after the antigen name, e.g., HPA-10bw. One possible reason why antibodies...
### Table 1. Platelet-specific alloantigen systems

<table>
<thead>
<tr>
<th>System</th>
<th>Antigen</th>
<th>Alternative names</th>
<th>Phenotype frequency* (%)</th>
<th>Platelet membrane glycoprotein</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
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<tr>
<td>HPA-1</td>
<td>HPA-1a</td>
<td>Zw⁺, P⁺</td>
<td>97.9</td>
<td>GPIIIα</td>
<td>T¹⁹⁶</td>
<td>Leucine³³</td>
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<td>HPA-1b</td>
<td>Zw⁺, P⁺</td>
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<td>HPA-2</td>
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<td>Koᵇ</td>
<td>&gt; 99.9</td>
<td>GPIbα</td>
<td>C²²⁴</td>
<td>Threonine³⁵</td>
</tr>
<tr>
<td></td>
<td>HPA-2b</td>
<td>Ko⁺, Sib⁺</td>
<td>13.2</td>
<td></td>
<td>T²²⁴</td>
<td>Methionine⁴⁵</td>
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<td>HPA-3</td>
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<td>Bak⁺, Lek⁺</td>
<td>80.95</td>
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<td>HPA-3b</td>
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<td>HPA-4</td>
<td>HPA-4a</td>
<td>Yulk⁺, Pen⁺</td>
<td>&gt; 99.9</td>
<td>GPIIIα</td>
<td>G¹²²⁶</td>
<td>Arginine³³³</td>
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<td>Yulk⁺, Penᵇ</td>
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<td>A¹²²⁶</td>
<td>Glutamine³³³</td>
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<td>HPA-5a</td>
<td>Br⁺, Zav⁺</td>
<td>99.0</td>
<td>GPla</td>
<td>G¹⁵⁶⁸</td>
<td>Glutamic acid⁵⁰⁵</td>
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<td>HPA-5b</td>
<td>Br⁺, Zav⁺, Hc⁺</td>
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<td>Lysine⁵⁰⁵</td>
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<td>Ca⁺, Tu⁺</td>
<td>0.7</td>
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<td>A⁺⁵⁶⁴</td>
<td>Arginine⁸⁹⁵</td>
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<td></td>
<td></td>
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<td>Alanine⁹⁷⁷</td>
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<td>C³²⁰¹</td>
<td>Cysteine⁶³⁵</td>
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<td>HPA-9</td>
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<td>Max⁺</td>
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<td>GPIIb</td>
<td>G²⁵⁰⁵</td>
<td>Valine⁹⁰⁷</td>
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<td>HPA-10</td>
<td>HPA-10bw</td>
<td>La⁺</td>
<td>&lt; 1.6</td>
<td>GPIIIα</td>
<td>G¹⁸¹</td>
<td>Arginine⁸²⁶</td>
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<td></td>
<td></td>
<td>A¹⁸¹</td>
<td>Glutamine⁸²⁶</td>
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<tr>
<td>HPA-11</td>
<td>HPA-11bw</td>
<td>Gro⁺</td>
<td>&lt; 0.25</td>
<td>GPIIIα</td>
<td>G¹⁹⁹⁶</td>
<td>Arginine⁶³³</td>
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<td></td>
<td>A¹⁹⁹⁶</td>
<td>Histidine⁶³³</td>
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<td>HPA-12</td>
<td>HPA-12bw</td>
<td>Ly⁺</td>
<td>0.4</td>
<td>GPIbβ</td>
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<td>Glycine¹⁵</td>
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<td>A¹⁴¹</td>
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<td>HPA-13</td>
<td>HPA-13bw</td>
<td>Sip⁺</td>
<td>0.25</td>
<td>GPla</td>
<td>C²⁵⁵¹</td>
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<td>Methionine³⁹⁹</td>
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<tr>
<td>HPA-14</td>
<td>HPA-14bw</td>
<td>Oe⁺</td>
<td>&lt; 0.17</td>
<td>GPIIIα</td>
<td>Δ AAG¹⁹²⁰⁻¹⁹³¹</td>
<td>Δ Lysine⁴⁴⁵</td>
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<td>HPA-15</td>
<td>HPA-15a</td>
<td>Govᵇ</td>
<td>74</td>
<td>CD109</td>
<td>C²¹⁰⁶</td>
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<td>HPA-15b</td>
<td>Gov⁺</td>
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<td>A²¹⁰⁸</td>
<td>Tyrosine⁷⁰³</td>
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<td>HPA-16</td>
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<td>Duv⁺</td>
<td>&lt; 1</td>
<td>GPIIIα</td>
<td>C¹⁷⁷</td>
<td>Threonine³⁴⁰</td>
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* Frequencies based on studies in Caucasians
against the “a” antigen have not yet been reported for many of the recently discovered systems is that the “b” allele is of such low frequency that “bb” homozygous individuals either do not exist or are extremely rare.

In Caucasian populations, the allele frequency for the majority of HPA systems is skewed toward the “a” allele and homozygosity for the “b” allele is below 3 percent. This places significant pressures on the blood services in the management of alloimmunized patients, as compatible RBCs and platelets are difficult to obtain for patients with antibodies against the “a” alloantigen. Allele frequencies vary between populations, e.g., HPA-1b is extremely rare or absent in the Far East, while HPA-4b does not occur in Caucasians (Table 2). These differences are important when investigating cases of suspected platelet alloimmunity in different ethnic groups.

Until the early 1990s, platelet typing was performed by serologic assays. These assays required the use of monospecific antisera, which were relatively uncommon, as the majority of immunized individuals produced HLA Class-I antibodies in addition to the platelet-specific antibodies. Therefore, typing that could be performed was limited, and many laboratories were only able to phenotype for HPA-1a. The publication of more advanced assays, such as monoclonal antibody-specific immobilization of platelet antigens (MAIPA) (Fig. 1), permitted more extensive phenotyping, but some antisera were simply not available.

With the advent of techniques such as immunoprecipitation of radioactive labeled platelet-membrane proteins, and the PCR, the molecular basis of the majority of clinically relevant platelet-specific alloantigen systems was elucidated. The molecular basis for all of the HPA alloantigen systems has been determined, and in all but one (Oea) the difference between the two alleles is based on a single nucleotide difference that results in a single amino acid substitution. Based on this molecular knowledge, a plethora of molecular typing techniques has been developed over the last decade, and these have largely overcome problems in platelet typing. One such assay is the PCR using sequence-specific primers (PCR-SSP). This is a fast and reliable molecular typing technique with minimal post-PCR handling. It has become one of the cornerstone techniques in HLA typing and is widely used for HPA genotyping (Fig. 2). Novel, high-throughput, DNA-based typing techniques with automated readout are under development and will be used in platelet immunology reference laboratories in the near future.

Knowledge of the genetic basis of platelet-specific antigens makes it possible to carry out molecular genotyping on whatever DNA-containing material is available, e.g., platelet typing using fetal DNA from amniocytes or from chorion villous biopsy samples. However, in the setting of first trimester fetal HPA typing, extreme caution is required to exclude possible contamination with maternal cells and consequent erroneous typing.

### Platelet Antibodies

Platelet antibodies can be targeted by different types of antibodies: autoantibodies, alloantibodies, isoantibodies, and drug-dependent antibodies.

#### Platelet Autoantibodies

Platelet autoantibodies cause the persistent thrombocytopenia (peripheral platelet count < 150 x 10^9/L) seen in idiopathic thrombocytopenic purpura (ITP). Autoantibodies bind to platelet antigens and cause the premature destruction of platelets in the reticuloendothelial system. The main targets appear to be the platelet membrane GP GPIIb/IIIa and GPIb/IX/V. Such autoantibodies bind to the platelets of all individuals, regardless of their HPA types.

ITP may be seen both in children and in adults, where it can follow an acute or chronic course. In adults, the condition typically has an insidious onset and runs a chronic course. Symptoms and signs are variable and include bruising, mucocutaneous bleeding, and frank hemorrhage. In general, serious bleeding symptoms are rare unless the ITP is severe (platelet count < 30 x 10^9/L). Adult chronic ITP has an

| Table 2. Platelet antigen frequencies in different populations |
|-----------------|---|---|---|---|---|---|---|---|---|---|
| 1a  | 1b  | 2a  | 2b  | 3a  | HPA-3b | 4a  | 4b  | 5a  | 5b  | Nak' |
| Caucasi ans | 97.5 | 30.8 | 99.8 | 11.8 | 86.1 | 62.9 | 100 | 0 | 98.8 | 20.7 | 100 |
| African blacks | 100 | 16 | 97 | 33 | 85 | 60 | 100 | 0 | 96 | 38 | 97.6 |
| Japanese | 99.9 | 3.7 | ? | 25.4 | 78.9 | 70.7 | 99.9 | 1.7 | 99.8 | 8.7 | 97 |
| Chinese | 99.9 | 0.15 | ? | 9 | 78.5 | 71.9 | 99.9 | 0.17 | 99.9 | 17.7 | 85.7 |
Fig. 1.  Monoclonal antibody-specific immobilization of platelet antigens. (1) A cocktail of target platelets; murine monoclonal antibody (MoMab) directed against the glycoprotein being studied, e.g., GPIIb/IIIa; and human serum is prepared in (a) the test serum containing anti-HPA-1a and (b) no anti-platelet antibodies. (2) After incubation, a trimeric (a) or dimeric (b) complex is formed. Excess serum and MoMab is removed by washing. (3) The platelet membrane is solubilized in a non-ionic detergent, which releases the complexes into the fluid phase, and particulate matter is removed by centrifugation. (4) The lysates containing the glycoprotein/antibody complexes are added to the wells of a microtiter plate previously coated with goat anti-mouse antibody. (5) Unbound lysate is removed by washing and an enzyme-conjugated goat anti-human antibody added. (6) Excess conjugate is removed by washing and a substrate solution is added. Cleavage of the substrate, i.e., a color reaction indicates binding of human antibody to the target platelets.
incidence of 58 to 66 new cases per million population per year in the United States and affects predominantly women of childbearing age. In children, the incidence of ITP appears to be lower than in adults, with published figures of between 4.0 and 5.3 per 100,000. The disorder also tends to run a different clinical course, with an acute and abrupt onset, often following a viral illness or immunization. The majority of children with the disorder require no treatment, and it resolves within 6 months. However, 15 percent of affected children develop a chronic form of ITP similar to that seen in adults.

The diagnosis of ITP usually relies on a typical history, blood count, and blood film. Although there are assays available for the detection of platelet auto-antibodies, these are not robust enough to establish the diagnosis of ITP alone. The direct platelet immunofluorescence test (PIFT) detects platelet-associated immunoglobulin (PAIg) levels, which are found to be increased in most patients with ITP. Unfortunately, the assay is not specific, since positive results can also be seen in nonimmune thrombocytopenias, for example secondary to sepsis. Assays for antibodies to specific platelet membrane GPs IIb/IIIa and Ib/IX are specific (90%) in ITP but less sensitive (50–65%), and currently their routine use in the diagnosis of ITP is not considered to be justified.

Testing for platelet autoantibodies may be of value when there is a combination of bone marrow failure associated with immune-mediated thrombocytopenia, in ITP patients refractory to first and second line treatments, in drug-dependent immune thrombocytopenias, and in rare disorders such as acquired antibody-mediated thrombosthenia.

Secondary immune thrombocytopenias can occur in association with underlying autoimmune disorders, e.g., systemic lupus erythematosus. Platelet autoimmunity is also frequently associated with B-cell malignancies, and autoantibody formation is not infrequent in the posthematopoietic stem cell transplantation period, during immune cell re-engraftment. In these situations, the presence of autoantibodies might contribute to refractoriness to donor platelets.

Platelet Alloantibodies

Detection of platelet alloantibodies

Tests for the detection of platelet-specific antibodies have evolved over the last 4 decades. Currently the most widely used assay is the indirect PIFT, but it is unable to distinguish between platelet-specific and HLA Class I antibodies. The MAIPA assay has become the gold standard for the identification of platelet-specific antibodies. This capture assay uses murine monoclonal antibodies specific for platelet GPs and can analyze complex mixtures of antibodies in patient sera. Third generation antibody detection assays, using purified or recombinant platelet GP, have also been developed; however, the sensitivity of these assays is not satisfactory for all alloantigen systems and some systems (e.g., HPA-15) are not included.

Clinical significance of HPA alloantibodies

HPA alloantibodies are responsible for the following clinical conditions:

- Neonatal alloimmune thrombocytopenia (NAIT), sometimes called fetomaternal alloimmune thrombocytopenia (FMAIT)
- Posttransfusion purpura (PTP)
- Refractoriness to platelet transfusions

NAIT

Definition

NAIT is defined as thrombocytopenia in the neonate (platelet count < 150 × 10^9/L) due to transplacental transfer of maternal platelet alloantibodies. However, thrombocytopenia can also occur in utero,
resulting in intrauterine death or intracranial hemorrhage (ICH).

**Incidence**

The estimated incidence of severe thrombocytopenia due to maternal alloantibodies is 1 per 1200 pregnancies in a Caucasian population, of which the majority are due to fetomaternal incompatibility for HPA-1a. NAIT is probably under-recognized in clinical practice.

**Pathophysiology**

NAIT is the platelet equivalent of the anemia seen in HDN. Maternal IgG alloantibodies against a fetal platelet alloantigen cross the placenta and bind to fetal platelets, resulting in their increased destruction in the reticuloendothelial system. Unlike HDN, up to 50 percent of cases of NAIT occur in the first pregnancy. In Caucasian women, the antibody most commonly implicated in NAIT (78–89% of cases) is anti-HPA-1a. Anti-HPA-5b occurs more frequently in pregnancy, but it tends to cause mild thrombocytopenia, and clinically significant NAIT is less common (6–15% of cases). The HPA-15 system, described a decade ago, has recently been found to have clinical significance, with HPA-15 alloantibodies being the third most frequently encountered antibodies. Rarely, NAIT can occur due to incompatibility for other HPA, HLA, or blood group ABO antigens. In non-Caucasian populations, other HPA alloantigens are more commonly implicated, for example HPA-4b in Orientals.

Approximately 2.5 percent of pregnant Caucasian women are HPA-1a negative and most of these women will carry HPA-1a positive fetuses, but only about 12 percent of these women will become HPA-1a alloimmunized. The ability to develop anti-HPA-1a is HLA class II restricted, with a strong association seen with HLA-DRB3*0101 positivity. As a negative predictive factor, the absence of HLA-DRB3*0101 for HPA-1a alloimmunization in HPA-1a negative women is > 90 percent, but its positive predictive value as a single marker is only 35 percent.

The pathogenicity of antibodies in an unselected population is highly variable. In a study including 26 cases of NAIT due to anti-HPA-1a, 9 of the 26 (34%) had severe thrombocytopenia; 35 percent (7 of 26 cases) had a normal cord platelet count, with a further 38 percent (10/26) having a “safe” platelet count of > 50 × 10⁷/L.

**Clinical features**

The typical infant with NAIT is full term and otherwise healthy, with a normal coagulation screen and isolated thrombocytopenia. The thrombocytopenia may be severe and unexpected, and it may occur in the firstborn child. Clinically, the neonate may present with petechiae, purpura, and/or ecchymoses. The most feared complication is ICH, which occurs in 14 to 26 percent of cases, resulting in death in 7 percent of affected cases and long-term morbidity with neurologic sequelae in 21 percent. Eighty percent of ICHs occur in utero, with 14 percent occurring before 20 weeks’ and a further 28 percent before 30 weeks’ gestation. This is consistent with the expression of platelet antigens from 16 weeks’ gestation and placental transfer of IgG antibodies that can occur from 14 weeks. There are also less common presentations of NAIT, including fetal hydrocephalus, hydrops fetalis, or recurrent miscarriages.

The differential diagnoses of thrombocytopenia in the newborn include infection, prematurity, intrauterine growth retardation, maternal platelet autoimmunity, and inadequate megakaryocytopoiesis (such as in the thrombocytopenia absent radii [TAR] syndrome).

**Laboratory diagnosis**

The diagnosis of NAIT requires the demonstration of maternal platelet alloantibodies that react against platelet-specific antigens present in the father and infant but not in the mother. Detection of maternal platelet-specific antibodies is usually carried out by two techniques, the indirect PIFT and the MAIPA assay using a panel of HPA-typed platelets. The serum of the mother is also tested against paternal platelets by both tests so that alloantibodies against low-frequency alloantigens and private antigens are not missed. The parents are also genotyped for the HPA-1,-2,-3, -5, and -15 alloantigens (the frequency of other alloantigens being comparatively low in the Caucasian population). It should also be noted that in up to 30 percent of cases of NAIT associated with fetomaternal incompatibility for the HPA-1a antigen, maternal HPA-1a antibodies may not be detectable. However, some authors have shown that the choice of MoAb used to capture GPIIb/IIIa in the MAIPA assay is critical and falsely negative results may be obtained if the epitope recognized by the MoAb is blocked by the human antibody. Others have reported that an increased serum:cell ratio is required to detect low levels of
antibody. Sometimes the antibodies that were not detectable at the time of delivery become detectable a few weeks or months after delivery.

Postnatal management

The postnatal management of NAIT is influenced by the degree of thrombocytopenia and clinical symptoms present. Any neonate with suspected NAIT who is bleeding or has a platelet count of \( < 30 \times 10^9/L \) should be transfused with compatible platelets to minimize the risk of ICH, without waiting for confirmatory laboratory tests.

Anti-HPA-1a and anti-HPA-5b are responsible for approximately 75 percent and 20 percent of cases of NAIT, respectively.\(^{17,24}\) Approximately 2 percent of blood donors are negative for both these antigens and can be easily identified using established platelet typing methods.\(^{25,26}\) It is possible to establish in blood centers a stock of HPA-1a-negative and 5b-negative platelet concentrates from donors who donate regularly, and these can be issued without delay for the treatment of suspected cases of NAIT before laboratory confirmation of the diagnosis.\(^{27}\) An alternative for platelets is random donor platelets, which, while more readily available, have been shown to have poorer responses, with one study of 36 cases of NAIT reporting a median increase in platelet count 24 hours posttransfusion of only \( 3.5 \times 10^9/L \).\(^{21}\) Washed maternal platelets have been found to be successful in the treatment of NAIT\(^{28}\) and may also be used. However, maternal platelets need to be collected by apheresis machines, washed to remove the maternal HPA antibodies, which may prolong the thrombocytopenia, and gamma-irradiated to prevent transfusion-associated graft-versus-host disease; all of which is time consuming and requires specialist equipment and facilities.

In the stable neonate, an alternative approach is to use IVIG at a dose of 1g/kg/day for 2 days. In a study of 12 cases of NAIT, a 75 percent response rate was reported. However, the increase in platelet count was delayed by 24 to 48 hours.\(^{29}\) In urgent situations, where antigen-negative platelets are not available and the neonate is bleeding, a combination of random platelets and IVIG should be given until compatible donor platelets become available.

Monitoring of neonates with NAIT is important in the postnatal period, irrespective of initial platelet level and clinical symptoms. The platelet count may continue to fall after birth, particularly in the first 48 hours, and thrombocytopenia may persist for up to 6 weeks postnatally (although 1–2 weeks is more usual). All neonates with NAIT should also undergo some form of cerebral imaging to exclude ICH.

Antenatal management

The realization that spontaneous ICH may occur in utero has led many to a search for methods of preventing serious antenatal bleeding. Unfortunately, 30 percent of cases of NAIT occur in the first pregnancy, and at present there are no reliable tests available to predict which women will become alloimmunized and which of those will have severely affected babies. What is well recognized is that mothers with HPA-1a alloimmunization and previously affected infants (particularly those with ICH) have a high risk of recurrence and poor outcome in subsequent pregnancies.\(^{30}\) These are the women targeted for antenatal treatment.

In women with a known history of alloimmunization and a previously affected pregnancy, HPA typing of the partner is important. This allows the risk of the fetus being HPA-1a positive to be assessed (i.e., all infants are affected when the father is homozygous for the pathogenic platelet antigen as opposed to 50 percent of infants affected in heterozygous cases). Where the father is heterozygous it may be useful to HPA type the fetus by chorionic villous or amniotic fluid sampling.

There is considerable experience in the antenatal management of FMAIT where there has been a previously affected pregnancy, but which is the optimal management remains controversial.\(^{31}\) The therapeutic options that have been explored are maternal administration of IVIG and/or steroids and fetal platelet transfusions. Early cesarean section alone is not considered to be effective in preventing antenatal or perinatal hemorrhage. For both approaches to antenatal management, fetal blood sampling (FBS) has been used for initial assessment of the fetal platelet count, usually at 20 to 22 weeks’ gestation, and for the monitoring of the effectiveness of treatment in the early studies.

Bussel et al.\(^{30}\) found maternal administration of IVIG to be successful, with no instances of ICH and most, but not all, infants, achieving a platelet count of greater than \( 30 \times 10^9/L \) at the end of pregnancy. The addition of steroids did not add to the effect of IVIG. However, ICH has been found during maternal treatment with IVIG,\(^{32}\) and a group of European centers...
treating 37 pregnancies only found success with the use of maternal IVIG in 7 of 27 cases (26%), and steroids in 1 of 10 cases (10%). It is difficult to understand why there was such a difference in the success of maternal administration of IVIG between these two studies. Relevant factors may include the methods used for assessing the success of treatment and the dose, timing, and type of IVIG used. The selection of cases may also be important.

A number of studies have shown the value of platelet transfusions given by cordocentesis in raising the platelet count, but the platelet count is raised for only a few days. A single pre-delivery transfusion may protect against bleeding at the time of delivery, but the fetus remains at risk of spontaneous ICH earlier in pregnancy. Weekly in utero platelet transfusions have been shown to be effective in preventing ICH in severe cases of FMAIT, but this approach is invasive.

In a recent study from the European Study Group for NAIT, the outcome of 56 fetuses receiving antenatal treatment for NAIT due to HPA-1a alloimmunization compared favorably with the outcome in previous pregnancies. Cases with a sibling history of antenatal ICH or severe thrombocytopenia (platelet counts of < 20 × 10⁹/L) had significantly lower pretreatment platelet counts than cases whose siblings had less severe thrombocytopenia or postnatal ICH. Maternal therapy resulted in a platelet count exceeding 50 × 10⁹/L in 67 percent of cases. None of the fetuses managed by serial platelet intrauterine transfusions (IUTs) suffered ICH after treatment started. However, the most serious complications encountered by the study cases were associated with fetal blood sampling (FBS). The results of this study support the use of maternal therapy as first line treatment for the antenatal management of NAIT. The observations of this study suggest that the commencement of maternal therapy can be stratified on the basis of the sibling history of NAIT. In two recent studies, concern regarding the safety of FBS led to the use of a less invasive treatment strategy involving maternal administration of IVIG without FBS for monitoring of the fetal platelet count, without an increased incidence of ICH.

Antenatal treatment appears to have the potential to improve the outcome of severely affected cases of NAIT, but there is little information on the long-term development of children who have been treated in utero.

**Routine antenatal screening**

Advances in laboratory diagnosis and antenatal management have drawn attention to the fact that the first affected fetus/neonate in a family is only recognized after bleeding has occurred, and this has raised the question of whether routine screening for NAIT would be advantageous.

An important initial question is whether to carry out screening antenatally or postnatally. The advantages of antenatal screening are that alloimmunized women can be identified during pregnancy, allowing time for antenatal intervention if it is agreed that this is appropriate. Even if no antenatal intervention is undertaken, the mode and timing of delivery can be planned to ensure minimal trauma to the baby's head and that compatible platelets are available, if needed. Postnatal screening can be achieved by simply carrying out a platelet count on a cord blood sample, but the major drawback of this approach is that the key objective of screening, to prevent morbidity and mortality from ICH, is unlikely to be achieved in the index pregnancy.

Although it is recognized that antenatal hemorrhage due to NAIT can produce devastating clinical effects, significant shortcomings exist in the knowledge about NAIT necessary for the introduction of an antenatal screening program. Further research is required on a number of issues, including the range of clinical outcomes in affected cases, the identification of factors useful for predicting severe disease, and the preferred option for antenatal management in women with anti-HPA-1a but no previous history of affected pregnancies.

**Posttransfusion Purpura**

A clinical case of thrombocytopenia developing 7 days after elective surgery, then spontaneously resolving 3 weeks later, was first described in 1959 by van Loghem and colleagues. The 51-year-old woman involved was found to have a strong platelet alloantibody, subsequently described as the first human platelet alloantibody, Zw. Two years later, a similar case was described by Shulman and colleagues, who coined the term posttransfusion purpura (PTP). In each case the same human platelet alloantibody was implicated (named Zw in the first case and anti-PlA1 in the case by Shulman, now known as HPA-1a).

**Definition**

PTP describes an acute episode of severe thrombocytopenia occurring 5 to 12 days after a blood
transfusion. It is usually seen in HPA-1a negative women previously alloimmunized by pregnancy or transfusion. The implicated transfusion is thought to induce a secondary immune response, boosting the production of HPA-1a antibodies and destruction of transfused donor platelets. At the same time, the patient's own platelets are also destroyed.

**Incidence**

PTP is considered to be a rare complication of transfusion, although the true incidence is unknown. A UK-based voluntary and confidential scheme for reporting serious hazards of transfusion (SHOT), set up in 1996, reported 43 cases in the first 6 years of the scheme, during which approximately 20 million blood components were transfused, giving an approximate incidence of 1 case in 465,000 transfusions. However, since the introduction of universal leukoreduction of blood components in 1999, there has been a reduction in the annual number of cases reported to SHOT.

As in NAIT, the susceptibility of HPA-1a negative individuals to PTP appears to reflect their ability to make HPA-1a antibodies, which is strongly associated with HLA Class II –DRB3*0101.

**Clinical features**

The typical patient is a middle-aged or elderly woman who has had a previous exposure to platelet antigens through pregnancy, transfusion, or both. The occurrence of PTP has also been reported in a small number of male patients. The time interval between the initial sensitizing event and the subsequent transfusion stimulating PTP is variable, with previous reports ranging from 3 to 52 years.

Clinically, the patient presents 5 to 12 days after transfusion with an acute severe thrombocytopenia (platelet count < 10 × 10^9/L) that has fallen from normal within 12 to 24 hours. Associated hemorrhage is very common and sometimes severe, with widespread purpura, and bleeding from mucous membranes and the gastrointestinal and urinary tracts. If untreated, the condition usually lasts between 7 and 28 days, although occasionally it may persist longer.

Implicated blood products include whole blood, packed RBCs, and RBC concentrates. PTP has also been reported following transfusion of plasma.

**Laboratory investigations**

A clinical diagnosis of PTP needs to be confirmed by the finding of platelet-specific alloantibodies in antigen-negative individuals. The majority of cases of PTP (80 to 90%) are associated with the development of HPA-1a antibodies, but other HPA antibodies have also been implicated, including HPA-1b, HPA-3a, HPA-3b, HPA-4a, HPA-5a, HPA-5b, HPA-15b, and Nak, and occasionally multiple antibodies are present.

Platelet-specific antibodies are detected using the MAIPA assay, which is able to resolve mixtures of antibodies, including HLA antibodies, that are often present in patients with PTP but do not appear to have a pathological role.

Other causes of a rapid onset of severe thrombocytopenia should also be excluded, such as disseminated intravascular coagulation (the coagulation screen is normal in uncomplicated cases of PTP), autoimmune thrombocytopenia, and drug-induced thrombocytopenia, e.g., heparin-induced thrombocytopenia (HIT). Rarely, thrombocytopenia may occur within 48 hours of a transfusion secondary to passively transfused platelet-specific alloantibodies from an immunized blood donor.

**Pathophysiology**

A rapid secondary antibody response (usually against HPA-1a) is stimulated by the RBC transfusion, with the result of acute thrombocytopenia about 1 week later. This time course of events is well established. However, it is still unclear why the patient's own antigen-negative platelets are destroyed. A number of hypotheses have been suggested:

- Transfused HPA-1a-positive platelets release HPA-1a antigen, which is adsorbed onto the patient's HPA-1a-negative platelets, making them a target for anti-HPA-1a. Support for this hypothesis comes from observations such as the elution of anti-HPA-1a from HPA-1a-negative platelets in some cases of PTP and the demonstration of the adsorption of HPA-1a antigen onto HPA-1a-negative platelets after incubation with plasma from HPA-1a-positive stored blood.

- The released HPA-1a antigen forms immune complexes with anti-HPA-1a in the plasma and the immune complexes become bound to the patient's platelets, causing their destruction.

- The transfusion stimulates the production of platelet autoantibodies as well as anti-HPA-1a. Evidence in favor of this mechanism is the detection of positive reactions of some PTP patients' sera from the acute thrombocytopenic phase with autologous platelets and the isolation
of autoantibodies that recognize calcium-dependent epitopes on GPIIb/IIIa during the acute phase.42
• In the early phase of the secondary antibody response, anti-HPA-1a may be produced, which has the ability to crossreact with autologous as well as allogeneic platelets.

Management

The main aim of treatment is to prevent morbidity and mortality associated with severe thrombocytopenia by shortening the duration of thrombocytopenia. A particular risk is ICH, which may cause early fatalities. Prompt treatment is therefore essential, to prevent this.

There have been no randomized controlled trials of treatment for PTP, and comparison of various therapeutic measures is complicated because of the occurrence of spontaneous remissions. Currently the treatment of choice is high-dose intravenous immunoglobulin (IVIG) (2g/kg given over 2 or 5 days), with rapid responses seen in about 85 percent of cases.43 Steroids and plasma exchange were the preferred treatments before the availability of IVIG, and plasma exchange, in particular, appeared to be effective in some, but not all, cases.

Platelet transfusions are usually ineffective in raising the platelet count, but they may be needed in large doses to control severe bleeding in the acute phase, particularly in patients who have recently undergone surgery before their response to high-dose IVIG. There is no evidence that platelet concentrates from HPA-1a-negative platelets are more effective than those from random donors; the dose probably is more important.

Prevention

PTP may recur, although this is unpredictable and occurs many years later. Patients with a previous episode should be issued a card to indicate that they need special blood products, and ideally future blood or platelet transfusions should be either autologous or from HPA-compatible donors.

The reduction in cases of PTP reported to the SHOT scheme since the introduction of universal leukoreduction in the UK suggests that leukoreduced blood products may be safe.

Refractoriness to Platelet Transfusions

Platelet transfusions are effective in decreasing hemorrhagic complications of severe thrombocytopenia. They may be given therapeutically to patients with active bleeding or prophylactically to patients with thrombocytopenia secondary to bone marrow failure. Serious spontaneous hemorrhage is unlikely to occur at platelet counts above $10^9 /L$, and this level has been widely adopted as a threshold above which prophylactic platelet transfusions are not required in an otherwise stable, nonbleeding patient.45 Although prophylactic platelet transfusions are standard practice for patients with bone marrow failure, no recent randomized controlled trials have compared therapeutic versus prophylactic transfusions in terms of incidence of hemorrhage and associated morbidity. Indeed, concerns about platelet refractoriness secondary to alloimmunization have led some to suggest lowering the platelet transfusion threshold to $5 \times 10^9 /L$.45

Definition

Platelet refractoriness is defined as the repeated failure to achieve satisfactory responses to platelet transfusions. This can be assessed clinically in a bleeding patient. However, where platelet transfusions have been given prophylactically, response is assessed by measuring the posttransfusion platelet count increment.

Various formulae have been devised to assess the platelet increment, including the percent platelet recovery and CCI. In practice, a 24-hour increment of $< 5 \times 10^9 /L$ on two or more occasions is a good indicator of refractoriness to random donor platelets.

Causes of platelet refractoriness

Refractoriness is due to the shortened survival of the transfused platelets in the recipient’s circulation, historically described in 20 to 60 percent of patients receiving multiple transfusions.46 The causes may be classified as immune or nonimmune, but in any individual patient there may be a multifactorial etiology.

Nonimmune clinical factors include disseminated intravascular coagulation (DIC), splenomegaly, and intravenous antibiotics (especially antifungal drugs such as amphotericin B).47,48 Fever has also been implicated in causing poor responses to platelet transfusions, although whether this is a reflection of sepsis, associated DIC, or antibiotic therapy rather than the temperature itself is unclear.49 Another unknown factor is whether platelet refractoriness may be due in part to inadequate dosage of platelets. Further studies...
are required to assess the optimal dosage of platelets, which may need to be adjusted according to a patient's blood volume.

The most common cause of alloimmune platelet refractoriness is HLA alloimmunization. Other immune causes include HPA alloimmunization, high-titer ABO antibodies in the recipient, platelet autoantibodies, and drug-related platelet antibodies.

HLA antibodies predominantly occur in women with a history of pregnancies and/or a history of multiple transfusions. If they are suspected as a cause for refractoriness, a combination of screening tests for both cytotoxic and noncytotoxic HLA antibodies should include the lymphocytotoxicity test (LCT) with either the lymphocyte or PIFT, or an ELISA-based method.

The role of platelet-specific antigens in platelet refractoriness is unclear. HPA antibodies occur at a frequency of 8 percent to 20–25 percent in various studies and are usually found in combination with HLA antibodies, although rarely they may occur in isolation. Most commonly, HPA alloimmunization is directed toward antigens with phenotypic frequencies below 30 percent. Some studies have suggested that there is no clear correlation between HPA antibodies and poor responses to platelet transfusions, but others have found that matching for platelet-specific antigens in those refractory to HLA-matched platelets may be beneficial.

Management

The management of platelet refractoriness first requires an assessment of possible nonimmune clinical causes. These should be corrected if possible and prophylactic platelet transfusions from random donors continued in the usual way. If poor responses to platelet transfusions persist, HLA antibodies should be sought in the patient's serum and, if they are present, platelet transfusions matched for the HLA-A and -B antigens of the patient should be used.

If poor responses continue, consideration should be given to ABO and/or HPA incompatibility. Testing for platelet-specific antibodies may identify HPA antibodies, and future platelet transfusions lacking the relevant antigen may be indicated. Platelet crossmatching may be helpful in cases where the platelet-specific antibodies have no obvious HPA specificity and the only way of identifying compatible donors is to find those who are crossmatch negative.

ABO incompatibility is an unusual cause of platelet refractoriness, seen usually when there are high-titer ABO antibodies in the recipient. ABO-identical platelet concentrates should be given to exclude this possibility.

Prevention

Leukoreduction of blood components has been shown to reduce the incidence of HLA alloimmunization and platelet refractoriness.

Drug-Dependent Antibodies

Drug-dependent antibodies are an important cause of shortened platelet survival that can often be overlooked, especially in hemato-oncology patients who may have thrombocytopenia for a variety of reasons. In some cases, drugs too small to elicit an immune response by themselves may bind as a hapten to platelet GPs in vivo. This haptenized platelet GP can trigger the formation of antibodies that only bind to the GP in the presence of the hapten. A classic example is quinine and its optical stereo-isomer, quindine. Typically, quinine-dependent antibodies are against GPIIb/IIIa, GPIb/IX/V, or a combination of both. Vancomycin has also been shown to induce the formation of IgG antibodies that bind specifically to GP IIb, IIIa, or both in the presence of the antibiotic. Similarly, the interaction of heparin with platelet factor 4 can cause antibody formation and lead to a drug-dependent thrombocytopenia. Evidence is also emerging implicating teicoplanin as a cause of immune-mediated thrombocytopenia.

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Transfusion-related acute lung injury (TRALI) is a serious clinical syndrome that is temporally associated with the transfusion of plasma-containing blood components. The syndrome typically occurs within 6 hours of transfusion. Approximately 80 percent of cases will resolve within 96 hours with supportive care. The syndrome has been associated with antibodies to WBC antigens and generation of biologically active mediators in stored cellular blood components. Appropriate laboratory investigation of TRALI can be crucial in confirmation of the clinical diagnosis, as well as in decisions regarding donor management.

Key Words: TRALI, TRALI pathophysiology and laboratory investigation, donor management—TRALI

Transfusion-related acute lung injury (TRALI) is a syndrome that is clinically indistinguishable from acute respiratory distress syndrome (ARDS). TRALI can occur within 6 hours of transfusion, but usually occurs within 1 to 2 hours. Symptoms and signs can include shortness of breath, difficulty breathing, hypoxia, fever, hypotension, and hypertension preceding hypotension. All plasma-containing blood components, including whole blood, packed RBCs, fresh frozen plasma, platelet concentrates, apheresis platelets, granulocytes, cryoprecipitate, allogeneic bone marrow, and IVIG, have been associated with TRALI. Although the pathogenesis of TRALI is not completely understood, it has been associated with antibodies to WBCs and with the infusion of biologically active mediators in stored cellular blood components.

Clinical Syndrome
In most cases of TRALI, clinical symptoms begin within 1 to 2 hours of completion of the transfusion. Symptoms can begin up to 6 hours after transfusion. There are rare case reports of mild symptoms beginning within 6 hours of transfusion with rapid resolution of symptoms and reappearance of more severe symptoms about 24 hours after transfusion. Signs and symptoms include fever, dyspnea, hypotension, hypertension followed by hypotension, and noncardiogenic pulmonary edema. Although any of these signs and symptoms can be seen in TRALI, not all of them will be present in every case.

Patients who are intubated, at the time of the reaction or shortly after the reaction begins, are described as producing copious quantities of frothy fluid from the endotracheal tube. The frothy quality of this fluid is secondary to its high protein content. The ratio of protein concentration in edema fluid to protein concentration in the blood in patients with noncardiogenic pulmonary edema is typically > 0.7, while the ratio is usually < 0.5 in cardiogenic pulmonary edema. Yost et al. analyzed the protein content of pulmonary edema fluid in seven cases of TRALI that occurred after liver transplantation. In all seven cases, the ratio of protein in the pulmonary edema fluid to protein in the blood was > 0.7.

The chest X-ray becomes abnormal at the time of the reaction. In recumbent patients, a patchy infiltrate may first appear in the dependent areas of the lungs. The infiltrate rapidly progresses to bilateral “white out.” This pattern is caused by pulmonary edema and is indistinguishable from the pattern seen in ARDS.

In approximately 80 percent of cases, the symptoms completely resolve within 96 hours of transfusion. Once the symptoms resolve, there are no chronic manifestations of TRALI. Fatal reactions occur in 5 percent to 10 percent of cases. Fatalities can occur acutely at the time of the reaction or after a protracted
course of mechanical ventilation. In the remaining cases, patients fully recover after intensive support and mechanical ventilation.

Differential diagnosis

The most important factor in the differential diagnosis of TRALI is to determine the nature of the pulmonary edema. The edema in TRALI is noncardiogenic in nature. Other forms of pulmonary edema include cardiogenic and volume overload. TRALI, or noncardiogenic pulmonary edema, can easily be confused with these other two types of pulmonary edema, particularly in critically ill patients and those who have received large volumes of intravenous fluids, including blood components.

Volume overload pulmonary edema is associated with other signs of fluid overload, including jugular venous distension and increased blood pressure. Cardiogenic pulmonary edema is associated with signs and symptoms of heart failure, including gallops, murmurs, or possibly evidence of myocardial ischemia/infarction on electrocardiogram. Additionally, laboratory tests for myocardial infarction and heart failure, including creatine kinase, troponin, and B-type natriuretic peptide, can aid in the differential diagnosis. All of these tests should be within normal limits in TRALI.

Incidence

TRALI is one of the most common causes of transfusion-related mortality. It has been reported as the third leading cause of transfusion-related mortality in the United States\(^2\)\(^2\) and the most common cause in the United Kingdom.\(^2\)\(^3\) An accurate estimate of the incidence of TRALI has been difficult to establish. Most of the published estimates of the incidence of TRALI are based upon calculating the number of cases of TRALI that occur at a specific institution over a period of time, compared to the number of components or patients transfused during the same period. These studies have led to estimates of one case of TRALI per 5000 components transfused\(^2\) and one case per 2400 patients transfused.\(^2\)\(^4\)

Although these studies give an estimate of the incidence of TRALI, there is a growing appreciation that TRALI may be under-diagnosed and underreported.\(^2\)\(^5\) A recent retrospective study examined the medical records of the recipients of blood components donated by a female donor who was implicated in a fatal case of TRALI.\(^2\)\(^5\) The implicated donor had a strong antibody to

the granulocyte 5b antigen (HNA-3a) and had donated apheresis plasma (600 mL) in a frequent plasma donation program. The medical records of 36 recipients of this donor’s plasma were reviewed by transfusion service medical directors for indications of TRALI associated with transfusion of the blood components. Reactions were classified as mild to moderate or severe. Severe reactions were defined as acute onset of pulmonary edema, or need for mechanical ventilation, associated with transfusion. Mild to moderate reactions were defined as dyspnea, and/or oxygen desaturation without overt clinical evidence of pulmonary edema, associated with transfusion. Eight severe reactions were identified in eight recipients, while seven mild to moderate reactions were identified in six recipients.

Only two of the eight severe reactions were reported to the transfusion service, while five of the seven mild to moderate reactions were reported to the transfusion service. Only two of the reactions (one mild to moderate and one severe) were reported to the blood collection facility. The two reactions reported to the blood collection facility were the fatal reaction that prompted the look-back and a mild to moderate reaction that occurred at the same institution during the initial investigation of the fatality. The observation that mild to moderate TRALI reactions were reported to the transfusion service more frequently than severe reactions may be explained by the fact that the mild to moderate manifestations of TRALI can clinically resemble febrile nonhemolytic transfusion reactions (FNHTR) that are routinely reported to the transfusion service.

This “look-back” study emphasizes the importance of clinical recognition of TRALI. Pulmonary symptoms that occur within 6 hours of transfusion should be reported to the transfusion service for further investigation, particularly when there is no other clinical explanation for the symptoms. After investigation by the transfusion service, probable cases of TRALI should be referred to the blood collection facility for donor testing and possible deferral of implicated donors, depending upon the results of the laboratory investigation.

The Antibody Hypothesis for TRALI

The first published case report of what was most likely TRALI was reported in 1951.\(^2\)\(^6\) During the next 30 years there were several case reports of noncardiogenic pulmonary edema associated with transfusion.\(^8\)\(^,\)\(^10\)\(^,\)\(^27\)\(^,\)\(^34\) These case reports attribute the
pulmonary edema to several different causes, including incompatibility of an undetermined nature,\textsuperscript{30} human leukocyte antigen (HLA) incompatibility,\textsuperscript{32} non-HLA leukoagglutinins,\textsuperscript{30} undefined granulocyte leukoagglutinins,\textsuperscript{34} and severe allergic pulmonary edema.\textsuperscript{8,16,31} Antibodies to HLA Class I and granulocyte antigens were associated with TRALI by Popovsky et al.\textsuperscript{2,17} In a series of 36 patients, granulocyte antibodies were identified in the serum of at least one implicated donor in 89 percent of cases. The presence of lymphocyte antibodies was determined, by reverse lymphocytotoxic crossmatch, in the serum of at least one implicated donor, in 26 of the 36 cases (72%). Specificity of the HLA antibodies was further characterized by lymphocyte panel testing in 17 of the 26 cases with HLA antibody. A clear HLA specificity was determined in 11 of the 17 cases (65%). The specificity of the antibody was determined to correspond to at least one of the recipient’s HLA antigens in 10 of the 17 cases.

Many of the early case reports of TRALI report the presence of granulocyte or HLA antibodies based upon a positive reverse granulocyte or lymphocyte crossmatch. The results of these tests can be misleading because of the presence of other antigen systems on these cells. This has the potential to result in incorrect interpretation of crossmatch results. An example of this is the granulocyte 5b antigen. Since this antigen is present on lymphocytes in addition to granulocytes, a positive reverse lymphocyte crossmatch may lead to the misinterpretation that lymphocyte or HLA antibodies are responsible for the TRALI reaction.

The technologies available to identify HLA antibodies have dramatically evolved in the last 10 years with the availability of solid phase assays such as flow cytometry and ELISA in most histocompatibility laboratories. Because these technologies are based upon isolation and adherence of HLA antigens to a latex bead (flow cytometry) or solid surface (ELISA), they are more specific for detection of HLA antibodies than tests based upon lymphocytotoxicity. Additionally, these tests do not depend upon the ability of the antibody to activate complement and cause cellular lysis.

A recent study by Kopko et al.\textsuperscript{7} investigated 16 cases of TRALI. This study examined donor-recipient pairs involved in TRALI reactions. Donors were tested for HLA and monocyte antibodies by flow cytometry, HLA antibodies via a lymphocytotoxic panel, and granulocyte antibodies by granulocyte agglutination (GA) and granulocyte immunofluorescence (GIF). Recipients were typed for HLA Class I and II antigens in all cases. If the donor(s) did not possess HLA or granulocyte antibodies, the recipient was evaluated for antibodies to HLA Class I and II antigens. In 14 of the 16 cases (87.5%), a correlation was identified between antibody present in the donor or the recipient and antigen in the other member of the donor-recipient pair. The identity of the antibody involved in these cases was HLA Class I (4), HLA Class II (5), HLA Class I and II (2), granulocyte (1), and monocyte (2). In the two cases where antibody was not identified, recipient samples were not available for antibody testing. Several recent case reports support the association of HLA Class II antibodies with TRALI.\textsuperscript{12,37–40}

Six of the 16 TRALI cases described above were further investigated for the ability of the sera containing antibody from one member of the donor-recipient pair to activate monocytes in the other member of the pair. The fraction of cells expressing the cytokines interleukin \( \alpha \beta \) (IL-1\( \beta \)) and tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)) or tissue factor were measured as an indicator of monocyte activation. Expression of the two cytokines and tissue factor were measured after exposure to autologous serum, control serum (serum from the donor of another blood component the patient received or ABO-matched control serum), or serum involved in the TRALI reaction. There was significantly increased expression of all three factors in the monocytes exposed to TRALI serum compared to autologous serum and control serum (\( p < 0.05 \)).

TRALI has been reproduced in an ex vivo animal lung model.\textsuperscript{41} In this model, pulmonary edema is produced after a latent period of 3 to 6 hours when rabbit lungs are perfused with 5b-positive granulocytes, antibody to granulocyte 5b, and rabbit plasma as a source of complement. If any of these three components is absent, pulmonary edema does not occur. The pulmonary edema produced in these experiments was noncardiogenic in nature. This was demonstrated by monitoring of pulmonary artery pressure, which displayed only a transient and moderate increase. Increased pulmonary vascular permeability was the cause of the edema.

In the antibody-mediated model of TRALI, antibody-coated leukocytes localize to the pulmonary microvasculature. Cytokine release by these leukocytes is thought to damage the vascular endothelium. Damage to the endothelium of the pulmonary microvasculature is thought to cause increased vascular permeability. This results in leakage of protein-rich fluid into the
pulmonary alveoli and results in pulmonary edema. This theory is supported by the findings of McCullough et al.,42 who have demonstrated that $^{111}$Indium-labeled granulocytes localize to the pulmonary microvasculature when transfused to a recipient with antibodies directed against the granulocytes.

A recent case report suggests that interaction of antibody with leukocytes may not be necessary for TRALI to occur.43 In this case, a 34-year-old woman who received two units of packed RBCs approximately 10 weeks after undergoing a lung transplant experienced a TRALI reaction. Pulmonary edema was present in the transplanted, but not in the native, lung. Antibodies to HLA-B were identified in the donor of the second unit of packed RBCs. No leukocyte antibodies were identified in the donor of the first unit of packed RBCs. The transplanted lung possessed the HLA-B antigen, while the patient did not. Since very few WBCs of donor origin would be expected to be present in the transplant recipient 10 weeks after transplant, and the pulmonary endothelial cells would be expected to be of donor origin, this case suggests that TRALI may be initiated directly through antibody interaction with endothelial cells.

The Biologically Active Mediator Model for TRALI

Biologically active mediators in cellular blood components, at or near the time of outdate, have been associated with TRALI.13 In this theory of TRALI, biologically active mediators or lipids accumulate during storage of cellular blood products, including packed RBCs and platelets. These agents enhance or prime neutrophil NADPH Oxidase. In this model, two events are required for a reaction to occur.44 In the first event, biologically active mediators are generated during physiologic stress. These stressors can include events such as trauma, infection, recent surgery, and massive transfusion. Generation of biologically active compounds, related to stress, is hypothesized to activate the pulmonary vascular endothelium and prime neutrophils. These actions are thought to cause sequestration of neutrophils within the pulmonary microvasculature. The second event consists of the infusion of biologically active mediators in a cellular blood component.

This model of TRALI is supported by a retrospective study performed by Silliman and colleagues.44 The records of ten transfusion recipients who experienced TRALI were assessed for the presence of a “first event” that would have predisposed them to develop TRALI. A control group of ten patients who experienced a febrile or urticarial reaction was also assessed for the presence of a “first event” prior to transfusion. All of the patients in the TRALI group had an underlying clinical factor that could have predisposed them to develop TRALI, while only two patients in the control group had such underlying clinical factors. Clinical factors thought to predispose a patient to developing TRALI included infection, cytokine administration, recent surgery, and massive transfusion.

This mechanism of TRALI has also been reproduced in an ex vivo animal lung model.45 In this study, rat lungs were first pretreated with lipopolysaccharide (LPS) to simulate sepsis. The lungs were perfused with one of the following solutions: buffered salt solution, 5% human plasma in saline, 5% supernatant from day 0 or day 5 platelets (both whole blood-derived and apheresis platelets), or lipid extracts from day 0 or day 5 platelets. Pulmonary edema (lung weight) and leukotriene B$_4$ levels (a measure of lung injury) were measured. Plasma from day 5 apheresis or whole blood-derived platelets, as well as lipid extracts from these components, caused increased pulmonary edema (p < 0.05) when the lungs were first treated with LPS compared to saline pretreated and saline perfused lungs. Pulmonary edema did not develop if the lungs were pretreated with saline instead of LPS. Lungs perfused with supernatant or lipid extract from day 0 whole blood-derived or apheresis platelets did not develop pulmonary edema. Leukotriene B$_4$ levels were increased in the lung perfusate (p < 0.05) only after pretreatment with LPS and perfusion of the lungs with 5% supernatant from day 5 platelets. Leukotriene B$_4$ levels were not measured after treatment with lipid extracts. Prestorage leukoreduction of platelets did not alter the pulmonary edema that developed in these experiments. Pulmonary artery pressure was monitored in these experiments to ensure the edema was noncardiogenic in nature. Significant, sustained increases in pulmonary artery pressure were not observed.

Silliman et al.46 recently published a study of 90 TRALI reactions occurring during a 4-year period at a single institution. The blood components implicated in these reactions were platelet concentrates (72), apheresis platelets (2), packed RBCs (15), and plasma (1). The first 46 reactions were analyzed in a nested case-control study. Patient and blood component data were compared in these cases to a control group of...
225 recipients of platelet concentrates. TRALI was not associated with patient age or gender, incompatibility of patient-donor blood groups, number of previous transfusions, or number and type of previous transfusion reactions. TRALI was associated with a diagnosis of hematologic malignancy ($p < 0.0004$) and cardiac disease ($p < 0.0006$). TRALI was also associated with increasing age of platelet concentrate transfused ($p = 0.014$). Pretransfusion and posttransfusion samples from the last 51 patients who experienced TRALI were analyzed for the accumulation of polymorphonuclear neutrophil leukocyte priming activity. There was significantly more priming activity ($p < 0.05$) in the posttransfusion samples compared to pretransfusion samples and controls.

**Pathophysiology of TRALI**

Although two seemingly dissimilar mechanisms have been associated with TRALI, it is possible that both mechanisms may be involved in the reaction. Activation of leukocytes with endothelial damage, increased vascular permeability, and resultant pulmonary edema are common to the antibody-mediated model of TRALI and the biologically active mediator model of TRALI. Additionally, look-back studies have shown that the presence of antibody in a donor is not sufficient to cause TRALI in all recipients, even if the recipient possesses an antigen corresponding to the infused antibody. These studies suggest that two events may be needed for TRALI to occur. Biologically active mediators have been demonstrated in cellular blood components, while they have not been routinely demonstrated in FFP. Since FFP has frequently been implicated in TRALI, biologically active mediators alone can’t explain all cases of TRALI.

An important point to remember when considering the two hypotheses for TRALI is that the differences in transfusion practice around the country and around the world may affect the findings in TRALI investigations. Although the author’s institution has reported identifying antibody in one member of the donor-recipient pair in the majority of cases, those data are possibly biased by our area’s transfusion practices. Hospitals supplied by the author’s blood center have exclusively received apheresis platelets for more than a decade. For the last 7 years, the apheresis platelets have been leukocyte reduced during the collection process. Additionally, the overwhelming majority (> 98%) of RBCs collected within the last few years have been prestorage leukoreduced. Therefore, if biologically active mediators are associated with nonleukoreduced platelet and RBC concentrates, it would be unlikely that a case of TRALI due to these substances would be found at the author’s center.

**Clinical Investigation**

The laboratory investigation of TRALI can be a frustrating and costly task. Cases of TRALI are often referred to the clinical laboratory with a large number of implicated blood components. Additionally, the laboratory is seldom provided data regarding when the implicated blood components were transfused relative to the reaction. Since most cases of TRALI occur within 1 to 2 hours of transfusion, this information is essential to the laboratory investigation of TRALI. Therefore, it is important to obtain this detail prior to initiating testing. This allows the laboratory to prioritize testing of implicated donors. The use of a testing algorithm in the laboratory investigation of TRALI can significantly reduce the cost of working up a case.

It is strongly recommended that donor-recipient pairs be investigated in TRALI cases. One of the most important steps needed to accomplish a complete laboratory investigation is to obtain the appropriate samples from the recipient as soon as TRALI is considered a possibility. If recipient samples for HLA typing and antibody studies are not drawn early in the investigation, they may not be available if they are needed to correlate results in the recipient with results from the donor(s).

A suggested testing algorithm is presented in Table 1. Testing should begin with female donors of blood components transfused within 2 hours of the reaction. Since testing for HLA antibodies is more readily available than testing for granulocyte antibodies, and HLA antibodies have recently been associated with TRALI more often than granulocyte antibodies, testing can begin with HLA Class I and II antibodies. If no HLA antibodies are detected, testing for granulocyte antibodies should be performed. The recipient should be HLA typed (Class I and II) to determine whether antibodies identified in a donor correspond to recipient antigens.

If female donors of blood components transfused within 2 hours of the reaction are negative for HLA and granulocyte antibodies, female donors of blood components transfused within 6 hours of the reaction should be tested for HLA antibodies, followed by testing for granulocyte antibodies. If all female donors
of blood components transfused within 6 hours of the reaction are negative for HLA and granulocyte antibodies, the recipient can be tested for these antibodies. This step will allow diagnosis of the approximately 10 to 15 percent of TRALI cases that are associated with antibodies in the recipient. If the transfusion recipient is at high risk of having leukocyte antibodies, due to a history of pregnancy or transfusion, it may be advisable to test the recipient for antibodies earlier in the course of the workup, particularly if the patient received a nonleukocyte reduced cellular blood component (i.e., packed RBCs or platelets).

If the recipient and the female donors of blood components transfused within 6 hours of the reaction are negative for HLA and granulocyte antibodies, male donors of blood components transfused within 2 hours of the reaction can be tested next. If the testing is still negative, male donors of blood components transfused within 6 hours of the reaction can be tested. At any point in the TRALI investigation, if antibody is identified in one member of the donor-recipient pair and it corresponds to antigen in the other member of the donor-recipient pair, further workup can be halted.

Currently, testing for monocyte antibodies and biologically active mediators is not commercially available. If testing for HLA and granulocyte antibodies is negative in the donor(s) and recipient, testing for these other factors associated with TRALI can be obtained through research laboratories.

### Treatment

Because TRALI occurs infrequently, there are no clinical studies of appropriate treatment after a reaction has occurred. The only treatments that can be recommended are supportive care for the patient’s symptoms, including oxygen support with or without mechanical ventilation, and fluid administration. The use of steroids for treatment of TRALI is anecdotal. Therefore, no recommendation regarding steroid use can be made.

One caution in the treatment of TRALI is the avoidance of diuretics. There are several case reports that describe worsening of the patient’s clinical condition and even fatality following administration of diuretics after a reaction.8,18 Since the pulmonary edema associated with TRALI is noncardiogenic, patients are not typically volume overloaded. The patient may be hypovolemic due to the underlying condition or secondary to the extravasation of fluid into the lungs. Therefore, administration of diuretics can lead to hypotension, decreased cardiac output, and decreased pulmonary capillary wedge pressure.8,18

### Donor Management Issues

One of the most important considerations regarding TRALI is donor management. Several different suggestions regarding donor management have been published. These include deferring all females from donating plasma for transfusion.49

### Table 1. Laboratory investigation of TRALI

**Recipient samples and testing**

1. When TRALI is first suspected, obtain serum/plasma and cellular samples (citrate anticoagulant)
2. Obtain HLA Class I and II typing on cellular samples or freeze cells for DNA for future testing
3. Freeze recipient serum/plasma sample for future testing (prereaction and postreaction samples, if available)
4. Separate and freeze liquid portion of remaining contents of bag, if available, for future testing

**Donor testing—HLA and granulocyte**

1. Obtain transfusion history including:
   a. Unit number of transfused component(s)
   b. Start/stop times of all components transfused
   c. Time of first symptoms of reaction
   d. Gender of donor of each of the components transfused within 6 hours of reaction symptoms
2. Test female donors of components transfused within 2 hours of reaction for HLA Class I and II antibodies
   a. If positive—correlate with recipient HLA typing (donor in step 4)
      i. If correlation exists—stop
   b. If negative—test donors (recipient in step 4) for granulocyte antibodies
      i. If positive—correlate with antigens in recipient (donor in step 4)
         1. If correlation exists—stop
      ii. If negative—proceed to next step
3. Test female donors of components transfused within 6 hours of reaction for HLA Class I and II antibodies (go to step 2a)
4. If recipient is at increased risk of having antibodies (multiparous or recipient of an organ allograft or multiple transfusions) and received a cellular blood component (go to step 2a)
5. Test male donor of components transfused within 2 hours of reaction for HLA Class I and II antibodies (go to step 2a)
6. Test male donors of components transfused within 6 hours of reaction for HLA Class I and II antibodies (go to step 2a)
7. Consider obtaining monocyte antibody testing (available through research laboratories only)

**Testing for biologically active mediators**

Testing should be considered (available through research laboratories only) if:
- Recipient received a cellular blood component with little plasma content
- Reaction occurred after transfusion of an autologous blood component
Review: transfusion-related acute lung injury

deferring multiparous females from donating plasma for transfusion, deferring multiparous females from plasma donation unless their serum has been tested for antibodies to leukocytes, and deferring donors implicated in a TRALI case, if they have been found to have antibody in their serum that corresponds to antigen on the TRALI patient’s leukocytes. Each of these donor management strategies would reduce the risk of TRALI in transfusion recipients. However, none of them would completely eliminate TRALI.

Deferring high-risk donors from donating plasma components would not eliminate the risks associated with the plasma in other blood components. A recent retrospective study correlated the volume of plasma in a blood component with the likelihood that a blood component would be implicated in a TRALI case. If the volume of plasma in a blood component correlates to the risk of TRALI from a blood component, then apheresis platelets would have a slightly greater risk of causing TRALI than plasma derived from a whole-blood donation. None of the deferral strategies that exclude donors based upon gender or parity include deferral from apheresis platelet donation. Additionally, very small volumes of plasma have been shown to cause TRALI. These deferral strategies would not prevent cases of TRALI secondary to the small volumes of plasma present in cryoprecipitate or RBC and platelet concentrates.

None of the donor management strategies would prevent cases of TRALI secondary to antibody in the recipient. Finally, none of the strategies would address the association of TRALI with biologically active lipids.

There are also obstacles to screening female or multiparous donors for antibodies to leukocytes. In order to screen for leukocyte antibodies, a test that could be performed on a large number of donors in a short period of time would be needed. Although screening tests for HLA Class I and II antibodies using flow cytometry or ELISA methodologies are currently available commercially, there are no such tests available for screening for antibodies to the granulocyte 5b antigen. Since this antibody has recently been reported in association with three fatal cases of TRALI, inclusion of a test for this antibody in a screening test would be ideal.

References


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Immune thrombocytopenic purpura (ITP) is an acquired disease in which autoantibodies to platelets cause their sequestration and destruction by mononuclear macrophages, principally in the spleen. If increased production of platelets by megakaryocytes does not compensate for platelet destruction, the number of circulating platelets decreases (thrombocytopenia), resulting in a characteristic bleeding tendency (purpura). While most children with the disease experience a relatively short and benign clinical course, ITP in adults often lasts more than 6 months (chronic ITP) and is resistant to conventional treatment (corticosteroids, intravenous immune globulin, or splenectomy). The goal of medical management is to increase the platelet count to a safe level, without the risks of bacterial infections associated with splenectomy or toxicity from prolonged corticosteroid therapy. Splenectomy increases platelet counts in hours to days in most patients with acute ITP, but nearly 50 percent experience recurrent thrombocytopenia 5 years postsplenectomy.

Key Words: platelets, thrombocytopenia, platelet antibodies, reticuloendothelial system, macrophage Fc receptors, splenectomy, intravenous immune globulin, IV Rh immune globulin, rituximab

Introduction

Immune thrombocytopenic purpura (ITP, idiopathic or autoimmune thrombocytopenic purpura) is an acquired bleeding disorder of children and adults that presents with thrombocytopenia as the only significant abnormal laboratory finding. There is neither a specific clinical nor a laboratory finding that reliably establishes a diagnosis of ITP. A diagnosis of ITP is established by excluding other potential causes of thrombocytopenia.

Classification

ITP is a heterogeneous disease with a wide range of clinical and laboratory presentations. Typically, patients are classified according to age at the time of diagnosis (ITP in children versus adults), but no one classification system is capable of segregating patients with this highly variable disease into categories without a significant number of exceptions. The goal of classifying patients is to be able to match a newly diagnosed patient with a group of other patients with similar findings for purposes of selecting an appropriate treatment and providing prognostic information.

Primary versus secondary immune thrombocytopenia

Conventionally, thrombocytopenias are divided into three major categories: those resulting from (1) abnormally decreased platelet production (e.g., aplastic anemia), (2) platelet sequestration (e.g., hypersplenism), or (3) abnormally increased platelet destruction (e.g., immune destruction). Immune (antibody-mediated) thrombocytopenia may occur as a complication of another disease (secondary thrombocytopenia) (e.g., HIV/AIDS, systemic lupus erythematosi, or a primary immunodeficiency syndrome) or as the only abnormal finding (primary immune thrombocytopenia). In the following review, “ITP” is intended to refer to primary immune thrombocytopenia.

ITP in children versus in adults

Typically, children with ITP present with an acute onset of petechiae and diffuse bruising approximately 4 to 8 weeks after a viral illness. As many as 85 percent of affected children experience an uneventful course and spontaneous remission within 6 months.1 In 2003, the International Childhood ITP Study Group reported the results of a prospective study of 2540 infants and children with newly diagnosed ITP, providing voluminous data on the clinical spectrum of childhood ITP.2 In this study, the incidence of intracranial hemorrhage, the most feared complication of ITP in
children, was only 0.17% during the first 6 months after diagnosis. In contrast to ITP in children, ITP in adults typically presents with an insidious onset; affects a preponderance of females, as seen in other auto-immune diseases in adults; and progresses to chronic ITP (persistent thrombocytopenia or a requirement for treatment 6 months after onset). This traditional profile of ITP in adults has been challenged by the results of a prospective study reported in 2003 for a population cohort of 245 adults with ITP in the UK.5 In this study, there were 134 females/111 males (1.2:1.0) and the majority of patients (63.3%) achieved remission (platelet count > 100,000/µL) and an additional 24 percent achieved a partial remission.

Pathophysiology

Harrington’s “thrombocytopenic factor”

While splenectomy has been recognized as an effective treatment for patients with ITP since it was first described in 1913,4 the modern concept of the pathophysiology of ITP began in 1951 when Harrington et al.5 reported “a thrombocytopenic factor” in the blood of patients with ITP Harrington infused himself and seven other nonthrombocytopenic subjects with whole blood or plasma from patients with “idiopathic” thrombocytopenic purpura and observed a rapid decrease in most recipients’ platelet counts. One patient’s plasma was fractioned by Cohn’s method and the thrombocytopenic effect was localized to the globulin fraction. This observation might have justified changing the name from “idiopathic” to “immune” thrombocytopenic purpura more than 50 years ago, but the designation “idiopathic” persists in common usage, even today.

Platelet autoantibodies

Autoantibodies that react with platelet membrane glycoproteins have been demonstrated in as many as 80 percent of patients with ITP.6 These antibodies react with glycoproteins IIb/IIIa (α₃β₃, integrin or CD41/CD61), GPIIb/IX, and others. As a consequence, autoantibody-coated platelets are phagocytized in the reticuloendothelial system (RES) via Fcγ receptors on mononuclear macrophages. Technically, the name “reticuloendothelial system” is a misnomer, with recognition that RES mononuclear phagocytes are neither reticular nor endothelial cells.7 However, the traditional concept that the spleen, liver, and bone marrow are functional sites for RES phagocytic activity is useful and is preserved in this review. Intuitively, one might expect that the severity of bleeding in patients with ITP would correlate with the presence of one or more of these antibodies, since their glycoprotein targets are integral to normal platelet function and hemostasis. However, current laboratory assays for platelet antibodies do not demonstrate a convincing correlation between specific glycoprotein targets and disease severity.6 Neither research-level nor commercially marketed assays for detecting platelet antibodies have sufficient specificity to be useful as diagnostic assays for ITP in clinical practice.8 An evaluation of the performance of Capture-P (Immucor, Inc., Norcross, GA), a commercially marketed kit for indirect platelet antibody tests, concluded that the method was not useful for establishing or excluding the diagnosis of ITP.8 This finding is consistent with the American Society of Hematology’s practice guideline, which concluded that current tests for platelet antibodies are neither necessary nor appropriate in the evaluation of ITP in children or adults.8 Recently, serologic reactivity of ITP autoantibodies has been further localized to epitopes on α₃β₃ or the amino-terminal portions of both GPIIbα and GPIIIα.10 McMillan et al.11 developed an immunobead assay to detect ITP antibodies reactive to GPIIb/IIIa or GPIb/IX that has a minimum specificity of 84.4 percent, raising the possibility that the long-awaited laboratory diagnostic assay for ITP may be forthcoming.

Polymorphic FcγR macrophage receptors

In ITP, autoantibody-coated platelets are removed from the circulation by Fcγ receptors on mononuclear macrophages, principally in the spleen. Among the three families of Fcγ receptors that have been recognized (FcγRI, FcγRII, and FcγRIII), there is considerable diversity of structure and function.12 FcγRI has a strong affinity for monomeric IgG. FcγRII and FcγRIII bind IgG only when it presents in an immune complex. FcγRII is polymorphic and two codominant alleles have been described, FcγRIIA-H131 and FcγRIIA-R131.12 In one study, the distribution of FcγRII receptors was skewed toward the FcγRIIA-R131 allotype, suggesting that Fc receptor polymorphisms may have a role in the pathophysiology of ITP or may be responsible for modulating the immune response.12 In another study, there was no significant difference in the distribution of FcγRIIA genotypes between ITP patients and controls, but there was a correlation between FcγRIIA genotypes and the response to
ITP autoantibodies have been identified in all IgG subclasses. The most prevalent IgG subclass detected among ITP antibodies is IgG1 (77%), but IgG2, IgG3, and IgG4 ITP antibodies have been identified. Further complicating the effort to identify a correlation between diagnosis or prognosis for ITP and a specific laboratory marker (i.e., antibody specificity, FcγR genotype, or the IgG subclass) is the observation that some patients with ITP appear to have oligoclonal platelet antibodies, whereas other patients have polyclonal antibodies.

Selected Treatments for ITP

In many persons with ITP, particularly children, thrombocytopenia resolves without specific treatment after a benign course lasting only a few days to a few weeks. Other persons may have sustained thrombocytopenia with life-threatening hemorrhages and a treatment-resistant clinical course lasting several years. For readers of Immunohematology with an interest in reading more than selected aspects of the treatment for ITP, I recommend the March 2002 issue of Blood Reviews, which contains 21 articles focused primarily on clinical management. The following brief comments are intended to address selected aspects of the treatment of ITP that have special relevance to immunohematologists and blood bank serologists.

The goal of current treatments for ITP is to decrease the synthesis of the platelet autoantibodies and/or decrease the rate of splenic destruction of autoantibody-coated platelets.

Splenectomy

The rationale for splenectomy in ITP is supported by studies that show elimination of both the white pulp (immune function) and red pulp (phagocytic function) are beneficial. Following splenectomy in ITP, antiplatelet antibodies decrease. This finding is not unexpected, since cultured splenic cells isolated from ITP patients synthesize IgG platelet antibodies. Also, splenectomy removes those mononuclear macrophages most likely to be responsible for destroying ITP autoantibody-coated platelets.

IVIG

A single infusion of a standard dose of IVIG (1 gram/kg) causes broad perturbations of both humoral and cellular immune function. However, the preponderance of evidence supports Imbach’s original explanation that the principal mechanism of IVIG’s beneficial effect in ITP is a consequence of “overloading and blocking of the reticuloendothelial system by IgG catabolism.” Fehr et al. showed that increased platelet counts following infusions of IVIG in four patients with ITP correlated with decreased clearance of IgG-coated RBCs, providing experimental evidence supporting Imbach’s hypothesis.

A single infusion of IVIG (1 gm/kg) is likely to be highly effective (> 85%) for increasing the platelet count by at least 30,000/µL in a previously untreated child or adult with ITP. Typically, the beneficial effect will last for 2 to 4 weeks, when a repeat infusion is needed to sustain the beneficial effect. However, if thrombocytopenia persists, the inconvenience of long infusions (3 to 5 hours), higher cost compared to IV Rh immune globulin (see below), and acute side effects (headache, flulike symptoms) often cause patients and physicians to consider alternative treatment programs.

Anti-D (IV Rh immune globulin)

In 1983, Salama et al. reported that microgram doses of anti-D (IV Rh immune globulin, IV RhIG) in D+ patients with ITP increased platelet counts in ITP to levels comparable to those reported after infusing gram doses of IVIG. Whereas IVIG-induced Fcγ receptor block results from random IgG molecules binding to mononuclear macrophages’ Fcγ receptors, IV RhIG-induced Fcγ receptor block in D+ patients leverages the larger and more numerous anti-D-coated D+ RBCs to compete with smaller and fewer autoantibody-coated platelets for Fcγ receptor binding. Assuming a representative platelet count of 10,000/µL and a normal RBC count of 5,000,000/µL in a patient with ITP, the competition of approximately 500 anti-D-coated D+ RBCs versus one autoantibody-coated platelet (500:1) favors phagocytosis of the RBCs and “RES blockade” of platelet sequestration and destruction. The efficacy of IV RhIG for increasing platelet counts in ITP has been confirmed by several studies (summarized in reference 22). In North America, treatment of patients with ITP with IV RhIG is increasingly popular compared to IVIG, because of the availability of an FDA-approved IV Rh immune globulin (WinRho SDF, Nabi, Boca Raton, FL), and its lower cost, ease of administration, and fewer acute side effects. WinRho is neither effective nor FDA-approved for treatment of ITP in D– patients. D– patients with ITP (approximately 15% of Caucasians are D–) may be treated with IVIG. Rarely, recipients of a standard dose have experienced acute intravascular hemolysis, with hemoglobinemia and hemoglobinuria.
No specific factor has been identified that predicts an acute hemolytic reaction, nor is there an explanation for the variable clinical responses observed after treatment with IV RhIG, raising the possibility that WinRho may have a variable avidity for different Rh phenotypes, reflecting the number of D antigen sites on the RBC membrane (dosage effect). This hypothesis was investigated using WinRho-coated RBCs of selected Rh phenotypes in direct hemagglutination and monocyte monolayer assays (MMAs), but neither serologic dosage effect nor differentiating reactivity of MMAs for RBCs with a double dose of D antigen was demonstrated.

Readers are reminded that while WinRho is commonly called “anti-D,” it is manufactured from pools of plasma collected from alloimmunized D− persons and different production lots may contain, in addition to anti-D, variable concentrations of anti-E, -C, -G and/or other alloantibodies. When WinRho is infused intravenously to treat ITP in a D+ patient, the IV bolus (50–75 µg/kg) is approximately 10 times the conventional intramuscular dose used for Rh immunoprophylaxis in D− women (300 µg, total dose). Therefore, serologic testing shortly after an infusion may detect multiple passively infused blood group alloantibodies, as well as the expected positive DAT. The presence of anti-D in the plasma of D+ ITP patients after an infusion of WinRho often raises the question, “Should D+ or D− RBCs be selected for transfusion, if required?” In this author’s opinion, the decision should be based on the immediate clinical priority. For example, if RBCs are required to treat anemia secondary to metrorrhagia in a woman with newly diagnosed ITP who remains thrombocytopenic, I favor transfusing D+ RBCs to react with any circulating WinRho and contribute to the immediate goal of increasing the platelet count. In contrast, if a patient is anemic, but no longer thrombocytopenic after an infusion of WinRho, I favor transfusing D− RBCs to support the immediate goal of correcting the anemia.

**Rituximab**

Rituximab (Rituxan, Genentech, Inc., San Francisco, CA; and Biogen Idec, Inc., Cambridge, MA) is a humanized IgG1/κ monoclonal anti-CD20 that is indicated for the treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive B-cell non-Hodgkin lymphoma. Typically, an infusion of rituximab is followed by a rapid decrease in B-lymphocytes with less myelosuppression than usually is observed with other immunosuppressive agents. As a consequence, rituximab is used increasingly “off label” to treat adults and children with chronic or refractory ITP. Reports of sustained remissions in many patients, together with the relatively selective B-cell immunosuppression, suggest that rituximab is likely to have an increasingly important role in the future management of ITP.

**Treating Helicobacter pylori infection**

In 1998, Gasbarrini et al. reported that eradication of *Helicobacter pylori* (*H. pylori*) in eight Italian adults with ITP and *H. pylori* gastric infections resulted in significantly increased platelet counts. This finding was supported subsequently by studies of *H. pylori*-infected persons with ITP in Italy and Japan. In contrast, in a carefully controlled study of *H. pylori*-infected ITP patients in New York, only one of 15 patients whose *H. pylori* infection had been eradicated experienced an increased platelet count, and that response was transient. Similarly, in a study in Spain, no significant improvements in platelet counts were observed in 56 patients with chronic ITP after eradication of *H. pylori* infection. The discrepancy in treatment outcomes remains unexplained and has resulted in divergent recommendations concerning the role of screening for *H. pylori* infection in patients with ITP. As one might anticipate, the authors of the study in Spain concluded that “there is insufficient evidence to include a search for *H. pylori* in the initial work-up of ITP patients.” Responding to this opinion, the authors of one of the studies in Italy wrote that “the data acquired to date still prompt us to consider the investigation and eradication of *H. pylori* in ITP patients as a simple, inexpensive tool in early management of the chronic disease. Even though the percentage of patients who are responsive to eradication treatment will be small, it should be mandatory [italics added] for the physicians to avoid the toxicity and discomfort that accompany long-term prednisone and other immunosuppressive treatments, also splenectomy.” In an editorial reviewing these diverse findings, McCrae concluded, “Should patients with ITP be routinely screened for *H. pylori*? At this point, probably not.”

For immunohematologists, a more intriguing question may be whether antibodies to *H. pylori* crossreact with antigens on platelets, causing ITP-like immune destruction. As one hypothesis to explain the inconsistent association of ITP and *H. pylori* infection, Michel et al. suggested that “the expression of various Lewis (Le) antigens by *H. pylori* isolates, and the
subsequent production of anti-Le antibodies, could play a role in ITP pathogenesis since platelets may adsorb Lewis antigens from the plasma. In fact, there are significant data to support this hypothesis, since Le\(^b\) functions as a receptor for \textit{H. pylori} in the gastric mucosa\(^{39-41}\) and Le\(^b\) may be adsorbed from plasma by platelet membranes.\(^{42}\) At the 1982 annual meeting of the American Association of Blood Banks, McGinniss reviewed the “ubiquitous nature of human blood group antigens” that are shared between RBCs, bacteria, viruses, and parasites.\(^{43}\) The relationship between Lewis antigens, RBCs, gastric mucosa, platelets, and \textit{H. pylori} was not recognized at that time. However, the \textit{H. pylori–ITP} issue would appear to present yet one more example of antigens that are shared among human blood cells and the diverse microbial agents that infect us.

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S. Gerald Sandler; MD, Professor; Medicine and Pathology, and Director; Transfusion Medicine, Department of Laboratory Medicine, Georgetown University Hospital, 3800 Reservoir Road, NW, Washington, DC 20007
The DAT is a test used to demonstrate in vivo antibody and/or complement coating of RBCs. Typically, the DAT is performed in test tubes; however, recently a number of commercially available tests using gel-filled microtubes have become available. Few data comparing the sensitivity of these test media are available. To compare the rate of detection of a positive DAT performed in test tubes versus in gel-filled microtubes and to assess the clinical significance of the results in patients undergoing evaluation of anemia, we tested 310 consecutive EDTA-anticoagulated blood samples from adult patients. The samples were analyzed using both the conventional tube technique and a gel-based assay (DiaMed®; Cressier sur Morat, Switzerland). Test results were expressed as either positive or negative. When a positive result by either technique was encountered, the treating physician was interviewed to determine whether the result warranted further patient investigation or treatment. In 268 out of 310 cases the DAT was negative by both methods. Of the 42 patients with a positive DAT, the test was positive by both methods in 18 patients. In the remaining 24 cases the DAT was positive by the gel test only. In all cases positive by both techniques the test result affected patient management. Of the 24 cases that were positive only by gel test, 3 were judged to be clinically significant. In this study, the gel test was more sensitive than the tube technique for performance of the DAT. However, the clinical significance of a DAT positive only by a gel test is doubtful. We believe that use of the gel-based DAT should be more extensively evaluated before it is adopted as a standard technique in general clinical laboratory practice. Immunohematology 2004;20:118–121.

**Key Words:** gel-based DAT test, tube DAT technique, clinical significance of a positive DAT

The DAT is a test used to demonstrate in vivo antibody and/or complement coating of RBCs. The principle of this test was first demonstrated in 1908 when rabbit erythrocytes, sensitized by goat anti-rabbit serum, were strongly agglutinated by the subsequent addition of rabbit anti-goat serum. However, it was not until 1945, when Coombs showed that blood group antibodies could be demonstrated in serum or on sensitized RBCs, that the test was adopted by clinical laboratories. The DAT is an essential test in the evaluation of autoimmune hemolytic anemia (AIHA). The DAT also has importance in the blood bank, where it is performed as part of the evaluation of hemolytic transfusion reactions.

Typically, the DAT is performed in test tubes; however, recently a number of commercially available tests using gel-filled microtubes have become available. Only limited data comparing the sensitivity of these test media and their clinical relevance are available. In this study, we compare the rate of detection of a positive DAT performed in test tubes versus in gel-filled microtubes and assess the clinical significance of the results in patients undergoing evaluation of anemia.

**Materials and Methods**

**Patients and samples**

We studied 310 consecutive blood samples sent to our hospital's Clinical Immunology laboratory for the DAT. The samples were collected in EDTA-containing tubes (Becton Dickinson and Co., UK) and were analyzed, using both the conventional tube technique and a gel-based assay, on the day of collection.

**DAT—tube technique**

DATs were performed by the classic tube technique using polyspecific antihuman globulin (AHG) serum (anti-IgG + anti-C3d; Gamma Biologicals, Houston, Texas). In this technique, RBCs were washed × 3 using NaCl 0.9% and resuspended to a 3–5% saline suspension. Two drops of polyspecific AHG were added to 1 drop of the washed cells, centrifuged for 1 minute at 900 × g, and examined macroscopically for agglutination. Positive samples were further tested...
with monospecific anti-IgG (Gamma Biologicals) and anti-IgA, -IgM, -C3c, and -C3d (DiaMed AG, Cressier sur Morat, Switzerland). Agglutination reactions were graded strongly positive (4+ and 3+), moderately positive (2+ and 1+), and weakly positive (W+), according to the manufacturer's directions.

**DAT—gel technique**

Gel tests were performed by adding 50 µL of a 0.8% RBC suspension in LISS (ID-Diluent 2) to the top of each microtube in a LISS/Coombs ID card (DiaMed AG). The cards were centrifuged at 910 rpm for 10 minutes, using the ID-Centrifuge 24S. All positive results (presence of agglutinated RBCs in the gel matrix) were re-examined using rabbit monospecific anti-IgG, -IgM, and -C3d (DC-screening monospecific Coombs sera, DiaMed AG). Negative reactions appeared as a discrete cell button at the base of the column.

**Further investigation of positive DAT tests**

When a positive DAT result was obtained by either the tube or the gel technique, the treating physician was interviewed by one of the investigators to determine the patient's diagnosis and whether the result warranted further patient investigation or treatment. In addition, laboratory data pertinent to possible hemolysis were abstracted from the patients' charts (serum bilirubin, lactate dehydrogenase [LDH], and, when available, haptoglobin levels and reticulocyte count). An isolated elevation of LDH was not considered to be indicative of hemolysis because of its lack of specificity for this diagnosis.

**Statistical analysis**

The sensitivity and specificity of the gel technique was calculated, using a result of the tube test, to be either truly positive or negative. From these calculations, the positive and negative predictive values were determined.

**Results**

Three hundred and ten samples were analyzed. Of these, 268 (86%) were negative by both tube and gel testing and 42 (14%) were positive by at least one test technique. Of the 42 positive samples, 21 (50%) were positive by gel and negative by tube testing, 3 samples (7%) were positive by tube testing and negative by gel testing, and 18 (43%) were positive by both tube and gel testing (Table 1).

<table>
<thead>
<tr>
<th>Number of positive and negative DATs (using polyspecific AHG) tests by tube and gel testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tube tests</strong></td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td><strong>Gel tests</strong></td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Of the 18 samples that were positive using both techniques, the IgG test only was positive in 11, while in 7 samples both IgG and C3 were found on the RBCs. No samples were positive for C3 only. Identical results were obtained in tube and gel tests. Of the 21 samples positive using gel testing only, 20 were positive for IgG and 1 was positive for C3 only.

The sensitivity of the gel test compared to the tube test using a polyspecific AHG reagent was 85 percent and its specificity was 93 percent. When a monoclonal IgG reagent was used, the sensitivity and specificity were 95 percent and 93 percent, respectively. The positive predictive value (PPV) of a positive gel test using the polyspecific reagent was only 46 percent while the negative predictive value (NPV) was 99 percent. For the monoclonal IgG reagent, the PPV and NPV were 47 percent and 99 percent, respectively.

**Clinical features of patients with a positive DAT**

The clinical diagnoses of the patients with a positive DAT by both gel and tube tests are listed in Table 2. Of note is that in patients with a positive DAT by tube and gel testing, the clinical diagnosis of most of the patients is one known to be associated with a positive DAT.

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>3</td>
<td>Systemic lupus erythematous</td>
</tr>
<tr>
<td>3</td>
<td>Immune thrombocytopenic purpura</td>
</tr>
<tr>
<td>1</td>
<td>Non-Hodgkins lymphoma</td>
</tr>
<tr>
<td>1</td>
<td>Monoclonal gammopathy</td>
</tr>
<tr>
<td>1</td>
<td>Hypersplenism, Hepatitis C positive</td>
</tr>
<tr>
<td>2</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Laboratory parameters of patients with a positive DAT**

Of the 18 patients with a positive DAT by tube and gel testing, 8 (44%) had at least 1 laboratory marker of hemolysis. However, of the 21 patients with a positive gel test only, 2 patients (9%) had positive hemolytic
parameters while none of the patients with a tube-test-only DAT had abnormal markers of hemolysis.

Effect of a positive DAT on further patient investigation or treatment

In 94 percent of the 18 patients with a positive DAT by tube and gel testing, the result elicited further diagnostic or therapeutic action, while an isolated positive gel test led to further diagnostic or therapeutic activity in only 9 percent of cases. None of the patients with a tube-test-only DAT had further tests performed nor received any treatment based on the result of the test.

Discussion

The use of gel-based microcolumn tests was introduced into the blood bank laboratory at the end of the 1980s for blood typing, antibody detection, and DATs. Gel tests have grown in popularity because of their increased accuracy and ease of use compared to classic tube tests. Furthermore, gel tests require smaller samples of RBCs and serum for testing, a distinct advantage when testing newborn and premature infants. Gel test systems also decrease the exposure of laboratory staff to potentially hazardous blood samples and breakable glassware. Finally, the test result obtained using gel test cards remains stable for up to 48 hours and can thus be saved for comparison or consultation after the test has been performed.

A number of studies have been published demonstrating the increased sensitivity of the gel test compared to tube testing in detecting RBC alloantibodies in patients’ sera. This increased sensitivity permits the detection of alloantibodies that would otherwise have remained undiagnosed, and has been shown to have definite clinical value in preventing potential hemolytic transfusion reactions in a number of cases.

Gel testing for the DAT compared to tube testing has been studied to a lesser extent, and the clinical relevance of a positive DAT by gel test is unknown. We performed the current study to determine the sensitivity, specificity, and positive and negative predictive values of one gel test (DiaMed) compared to traditional tube testing, which until now has been considered the standard test system. We also sought to assess the immediate clinical relevance of a DAT positive by either technique by determining the treating physicians' response to the test result in terms of ordering further diagnostic tests or instituting therapy based on the test result. Our results show that of 42 samples positive by either technique using polyspecific antihuman globulin, 18 (43%) were positive by tube and gel testing, while 21 (50%) were positive by gel testing only. Three samples (17%) were positive by tube testing and negative by the gel test. Thus the sensitivity of the gel test was 86 percent. When monospecific reagents (anti-IgG and anti-C3) were used, the sensitivity increased to 95 percent for IgG and decreased to 78 percent for C3. These results are similar to those of Tissot et al., except that in their study the sensitivity of the gel test using anti-C3 was only 16 percent. This may be significant because it is known that in patients with AIHA, hemolysis is more severe when both C3 and IgG are present on the RBCs.

Our study has demonstrated that the gel test is highly specific compared to the tube test: 93 percent using polyspecific and anti-IgG reagents, and 99 percent using anti-C3. The results are concordant with those of Tissot et al., but differ from those of Nathanlang et al. The latter group compared gel and tube DAT tests in newborns with suspected fetal-maternal ABO incompatibility and in adults with known AIHA and found a sensitivity of 94 percent and a specificity of 85 percent. When only the adults in the study are considered, the sensitivity and specificity were 100 percent and 80 percent, respectively. These differences may be accounted for by the fact that Nathanlang et al. studied a different patient population than ours: all of their adult patients had known AIHA and thus would be more likely to have a positive DAT by any technique, compared to our patients, who were undergoing an evaluation for anemia whose cause was undetermined at the time of DAT testing. This difference in patient population also explains the lower PPV of the gel test in our study (46%) compared to 94 percent among the adult patients in their study.

When we analyzed the clinical relevance of the positive DAT, we noted that in 17 of the 18 patients (94%) with a positive DAT by both techniques, the result impacted clinical decision making. By contrast, in only 2 of the 21 patients (9%) with a positive DAT by gel testing only did the result lead to further relevant clinical activity. A similar trend was found when laboratory markers of hemolysis were examined in patients with a positive DAT. When the DAT was positive by both techniques, hemolytic parameters were observed in 74 percent of patients, while only 9 percent of patients with a gel-only positive DAT had other laboratory evidence of hemolysis.
These results suggest that the gel test is more sensitive than the conventional tube test for performance of the DAT. The relatively low number of patients with a gel-only positive test having clinical or laboratory evidence for hemolysis suggests a high falsely positive rate for the gel test. However, we cannot exclude the possibility that some patients in our study who had a positive gel test may develop hemolytic anemia or another disease related to a positive DAT in the future. Furthermore, in this study we did not take into account the strength of agglutination obtained in the gel test. This was because, at the time that this study was performed, our hospital’s immunology laboratory reported DAT test results as “positive” or “negative” and we postulated that treating physicians were likely to make clinical judgements on this basis without considering the strength of a positive test result.

In conclusion, we believe that aspects of the gel-based DAT, such as the correlation of strength of agglutination with evidence for hemolysis, should be more extensively evaluated before it is adopted as a standard technique in place of conventional tube testing in general clinical laboratory practice.

References

Na’ama Paz, MD, Blood Bank, Meir Hospital, Israel; Dganit Itzhaky, PhD, Immunohematology Laboratory, Meir Hospital; and Martin H. Ellis, MD, Blood Bank, Meir Hospital, Kjar Saba 44281, Israel, and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel (corresponding author).
Letter to the Editors

An unusual antibody reacting with pre-diluted 0.8% reagent RBCs and with 0.8% older (aged) RBCs prepared at the time of testing

A 58-year-old, nontransfused, group O male presented at hospital for pretransfusion testing prior to surgery. The preoperative screens and initial panel were performed using a gel test and reagent RBCs pre-diluted with LISS to 0.8% by the manufacturer (Ortho-Clinical Diagnostics, Inc., Raritan, NJ). All cells tested, except the autocontrol, were 2+ positive. The patient’s DAT was negative. The hospital had also performed tube LISS crossmatches and screens but observed no reactivity.

The sample was referred to our red cell reference laboratory for investigation of a potential antibody to a high-frequency antigen reacting only by gel test. As is our standard testing protocol, the patient's serum sample was tested with a three-cell screen plus an autocontrol using the following: LISS-antiglobulin (ImmuAdd; Immucor Inc., Norcross, GA), ficin antiglobulin technique (GammaZyme-F; Gamma Biologicals Inc., Houston, TX), and a gel test using RBCs diluted at the time of testing with Diluent 2 (MicroTyping Systems Inc., Pompano Beach, FL).

No reactivity was observed with any of the test methods. This excluded clinically significant alloantibodies to common RBC antigens and antibodies to high-frequency antigens reacting by routine methods currently used by our laboratory.

The hospital had tested the sample using a gel method and pre-diluted RBC suspensions. Since our center was also using Ortho’s pooled pre-diluted 0.8% RBCs for high-volume donor screening, we tested this patient’s serum against these pooled RBCs in gel and also observed a 2+ reaction. Another lot number of the pooled pre-diluted cells was available and the 2+ reaction was reproduced. This latter example was washed (× 3) free of diluent with 0.9% normal saline and retested. There was no change in reactivity. This demonstrated that the reactivity was neither diluent-dependent nor caused by something that could be washed from the RBC surface.

Since this case was referred to us as a possible antibody to a high-frequency antigen, we prepared a selected panel of RBCs negative for high-frequency antigens from our liquid inventory. We prepared the RBCs as 0.8% suspensions in Diluent 2 and tested the RBCs with the patient’s serum immediately by the gel test. To this point, the only reactive RBCs had been those that had been pre-diluted by the manufacturer. We now had some 2+ reactions and some negative results. We then observed that the only reactive RBCs were greater than 3 months old at the time of testing.

We then tested older (aged) examples of previously nonreactive RBCs, side by side, in the gel cards and found that the older examples now gave us up to 2+ reactions. The DAT on these older RBCs was negative using gel-IgG cards.

We then decided to “age” some reagent RBCs at 37°C overnight in a water bath and test them the next day. The so-called “aged” cells gave 2+ reactions and their “fresh” counterparts were nonreactive.

The patient’s serum and ten random male donor sera were tested against two 0.8% pre-diluted, DAT-negative screening cells by the gel technique. The patient’s serum reacted 2+ with both reagent RBCs and all ten of the donor sera were nonreactive.

These same donor sera were then tested in parallel with the patient’s serum against DAT-negative RBCs from one donor. The single-source RBCs were tested “fresh,” i.e., 2 weeks from expiration, 3 months postexpiration, and 6 months postexpiration. The three RBC samples were diluted to 0.8% suspensions and tested immediately using the gel test. The ten donor sera were nonreactive with all cells tested but the patient’s serum reacted as expected. No reactivity was observed with the “fresh” RBCs, a 1+ reaction was observed with the 3-month aged RBCs, and a 2+ reaction with the 6-month aged RBCs.

The conclusion was that a constituent of the diluent used by Ortho to predilute their RBCs was...
producing an immediate effect on the RBC surface that was detected by this patient’s serum, an effect not apparent with other commercially prepared RBCs until they were aged during storage. This effect was demonstrable by gel test.

Although the unusual antibody observed in this case had no relevance to treatment of the patient, it is prudent to share abnormal findings with our colleagues. Sharing such results enables us to better understand the advantages and disadvantages of different antibody screening techniques.

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Attention SBB and BB Students: You are eligible for a free 1-year subscription to Immunohematology. Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to Immunohematology, P.O. Box 40325, Philadelphia, PA 19106.

Attention State Blood Bank Meeting Organizers
If you are planning a state meeting and would like copies of Immunohematology for distribution, please contact Mary McGinniss, Managing Editor, 4 months in advance, by phone or fax at (301) 299-7443.
Letter to the Editor-in-Chief

Looking back at the history of the journal

It’s hard to believe that 20 years have passed since you asked me to write the first article for the very first issue of your new journal, Vol. 1, No. 1, 1984, *Immunobematology*. Each year, you and your staff have managed to produce a prodigious series of journals at a price which was, and still is, affordable to all! The journal has covered a wide range of topics, all practical, timely, and important to the field of immunohematology. In addition, the journal has attracted an editorial board composed of individuals, well known in the field, who have donated their time and expertise in making it a quality peer-reviewed publication. As the needs of blood bank technologists, physicians, and educators have changed to reflect the advances and redesign of transfusion medicine, so has the focus of the journal kept pace over the past 20 years. *Immunobematology* has come a long way and has become a favorite journal of all blood bankers! It was the first of its kind to provide current and timely articles on practical issues for blood bank serologists and educators. In the beginning, and now, the journal continues to provide wonderful review articles, original scientific studies, unusual serologic cases, and literature reviews. The straightforward presentations of serologic case studies make it an invaluable resource! The actual serologic characteristics of certain antibodies reported in your journal has made it a convenient resource for blood bank technologists to recognize unusual antibodies. In addition, your willingness to guide and nurture first-time authors has made the journal a great success!

Even after 20 years, *Immunobematology* remains a well-balanced, timeless journal, designed for easy reading, and infused with a richness of tradition! Thank you for a wonderful journal under your leadership! The commitment and dedication of all those who have contributed to this journal have made this publication well acknowledged and admired by everyone in the field. I would like to personally thank you and applaud the journal for its many years of service to the profession.

Denise Harmening, PhD, MT(ASCP)
Department of Medical & Research Technology
University of Maryland School of Medicine
Baltimore, MD 21201

**IMPORTANT NOTICE ABOUT MANUSCRIPTS FOR IMMUNOHEMATOLOGY**

Mail all manuscripts (original and 2 copies) to Mary H. McGinniss, Managing Editor, 10262 Arizona Circle, Bethesda, MD 20817. In addition, please e-mail a copy to Marge Manigly at mmanigly@usa.redcross.org
Letters From the Editor-in-Chief

The second 20th anniversary issue!

This second issue of Immunohematology truly celebrates 20 years of publishing by bringing you four great reviews, one original paper, and one scientific letter to the editor. The guest editor of this Platelet Review Issue is Dr. Scott Murphy, medical director of the Penn-Jersey Red Cross in Philadelphia, Pennsylvania, and senior medical editor of Immunohematology. Dr. Murphy has invited recognized experts in the field who have written reviews for the reader that will be of great value for a long time to come.

In this issue you will also find a letter from Dr. Denise Harmening who looks back at the history of the journal. She was the first author in the first issue of the journal, published 20 years ago.

My sincere apology

In the last issue I neglected to explain that Christine Lomas-Francis, MSc, guest editor of the first 20th anniversary issue of Immunohematology, is the technical director of the laboratory of immunohematology at the New York Blood Center and Technical Editor of Immunohematology. I hope the readers and Christine will accept my sincere apology.

Memories from the past!

In this issue and for the rest of the issues in 2004, we are publishing excerpts from early issues of the Red Cell Free Press. See Special Section. They represent some of the interesting case studies, methods, and fun things, such as a crossword puzzle, that were published in the newsletter in the late 1970s. You will find the answer to the crossword puzzle at the end of this issue. The Red Cell Free Press was the precursor of Immunohematology, started in 1984 by Sandy Ellisor, Marion Reid, and Helen Glidden at the American Red Cross. It was designed to communicate and interact with the ARC Reference Laboratory staff.

What is next?

The September issue of Immunohematology will be a very exciting issue and one to look forward to. Sandra Nance will be the guest editor and the topics of the invited reviews will be: “Drug-induced hemolytic anemia: an update,” by Dr. George Garratty; “What do you do when all units are incompatible: clinical aspects,” by Dr. Geralyn Meny; “What do you do when all units are incompatible: serologic aspects,” by Pat Arndt and Sandy Nance; and “Evaluating patients with immune hemolysis,” by Dr. Larry Petz. The September issue will be outstanding!

Remember, we are always ready for more papers from original studies.
**Lan and Gonsowski**

We were referred a blood sample on a patient who was admitted to a hospital for TUR surgery. The blood center suspected the patient’s serum contained an antibody to a high frequency antigen. In our laboratory, the routine antibody workup was performed and a battery of high incidence negative cells tested. Both Lan negative and Gn(a−) cells were found non-reactive with this patient’s serum, whereas all other cells were strongly incompatible in the antiglobulin phase of testing. After resolving that this patient’s antibody was anti-Lan and that he was Lan negative, we investigated the Gn(a−) red blood cells and serum from Mrs. Gonsowski.

Red cells from Mrs. Gonsowski drawn on two different occasions were tested with several examples of anti-Lan. No reactivity occurred. Mrs. Gonsowski’s serum, which was known to contain anti-D as well as high incidence antibody, did not agglutinate three Rh(D) negative Lan negative red cell samples. Mrs. Gonsowski’s serum was then absorbed onto a random group O, Rh negative (rr) red cell sample and an eluate prepared from the absorbing cells. Lan specificity was once again obtained.

A fresh blood sample on Mrs. Gonsowski was kindly sent to us for repeat testing. The above results were duplicated, and other investigators have also verified these results. We are therefore noting that anti-Gn, which was reported earlier as a new antibody independent of other blood group systems, is really anti-Lan and that Gn(a−) cells are Lan negative.


**Obtaining Cells from a Clot for Absorption, Elution, etc.**

Although we request serum and an anticoagulated specimen, hospitals often send just a clotted specimen. To obtain sufficient cells for absorption, elutions, etc., we have found the following procedure to be useful:

- Cut the top off the filter of a Recipient Set. We use Fenwal HB-98D. Discard the drip chamber and attached tubing.
- Pour the clot into the filter chamber, with the spike sitting in a 15 cc tube.
- Mash the clot around the filter with applicator sticks and squirt saline to wash as many cells as possible into the tube.
- Wash the cells until free of hemolysis.

We think this is great for salvaging a lot of cells with a minimum amount of trouble.

*Beverly Pohl*

Crossword Puzzle Number 4

ACROSS
1. Ouchterlony technique is an agar gel ______ method.
10. White cell responsible for humoral immunity (2 words).
15. Antibody in serum of Claas and McLeod.
16. Antigen complex absent or weak on ABH\textsubscript{null}, A1B cells and Oi cells.
22. Complement component bound before C3 in classical pathway.
23. AutoAnalyser.
24. This form of polyagglutination is specifically agglutinated by S. Scleria.
25. Highfalutin Sd antigen.
28. Unusual, uncommon or low incidence antigen.
30. Serum and plasma mixed together will _______.
33. Group A secretor gene.
35. Gene controlling secretion of Lewis substance in body fluids.
36. Stuart Prower factor.
37. Sc:1 antigen.
41. RES red cell destruction.
45. Immune response genes.

46. An animal used to produce antihuman antibodies.
47. An antigen which non-specifically stimulates lymphocytes.

DOWN
2. Antibody reacting only with cells possessing I and B antigens.
3. A procedure for demonstrating immunoglobulin on the surface of B cells.
4. Symbol for the Duffy blood group system.
5. The site used to obtain blood samples from the immediate neonate.
6. Another symbol for HD or Pr.
8. A female gamete.
9. Of no commercial value.
11. A type of polyagglutination.
12. The process of separating antibody from its antigen.
13. Bilirubin is ________ to albumin for transport within the body.
17. Low frequency antigen in the MN system.
18. First half of the name for the biphasic hemolysin found in patients with PCH.
20. The Langereis or So\textsuperscript{+} blood group system.
26. This can affect antigen and antibody strength.
27. Rh\textsuperscript{w1}
28. Rheumatoid arthritis.
29. Spleen, liver, et al.
31. Isotonic, low ionic strength solution used in immunohematology.
32. A system of adjacent genes on a chromosome of which one gene, the operator gene, controls the activity of the structural genes.
34. Y and X genes control this (X-rated).
38. Low frequency antigen in the MN blood group system described in Nature 172:688(1953).
39. A relative of gastropod mollusk that can be used as a source of anti-A.
40. A major city in the USA.
42. A third generation method to test for HB,Ag.
43. Automated reagin test.
44. Usual site of venipuncture.

Marion Reid
For solution, see this issue, page 133.
**BOOK REVIEW**


No scientific topic has been more in the press and in legislative hearing rooms than stem cells. Behind the news-catching events of the last 5 years, there has been an amazing amount of research into the mechanisms of action of stem cells: (1) from differentiated tissue, where the stem cells tend to repair or repopulate the organ where they reside; (2) from primitive or embryonic tissue that has not yet made the journey of differentiation; and (3) from cells that appear to be able to transdifferentiate from the organ where they reside to other tissues. This phenomenon of plasticity is under intensive research by investigators studying readily available adult stem cells, for example, those in the blood and bone marrow, and their ability to become a stem cell for another organ of the body.

This book begins with a historical perspective on stem cells and walks the reader straight to the knowledge revolution of the last few years. It accomplishes this with information instead of passion, with clear studies and data instead of polarized points of view. This book helps place into context the much-publicized and controversial studies highlighted by the news media.

The first chapter, by the editor, gives an important overview of the path of differentiation and some possible side trips of nature. It also offers clear definitions of terms that make the information in the rest of the book more understandable. Each chapter author has a depth of knowledge in his or her field, yet they are all careful to include examples of practical use of the information, either in further basic research or in possible treatment applications.

Methods of growing and isolating embryonic stem cells are covered in Chapters 2 and 3. Chapter 4 traces the journey from embryonic stem cell to functional, differentiated tissue architecture. Chapter 5 explains germ cell interactions and combinations as they move on their way to becoming embryonic stem cells or supporting their growth.

Chapter 6 covers the cloning of mammalian cells, with a description of the difficulties of the processes as well as the observed outcomes of producing either new cloned organisms or sources of cells for repair of the current organism.

In Chapter 8, repair and wound healing are described first in the amphibian model, where these mechanisms are extensive. This is followed by two examples of human repair systems in the next two chapters: dermal repair and reconstruction of skin; and bone marrow mesenchymal cells for reconstituting mesodermal tissues such as bone, cartilage, muscle, tendons, and ligaments, as well as bone marrow stromal cells. Normal and abnormal hematopoietic stem cell lineages are explored from the starting point of the hematopoietic stem cell. The authors also discuss use of the hematopoietic stem cell as a means of rescue, treatment, or both of leukemias and lymphomas.

Beginning with Chapter 16, the book takes us first through the development of organ systems, then through the development of disease, with suggestions for areas of research that may lead to treatment using stem cell-based or augmented strategies. The coverage of neural, cardiovascular, and hepatic organs is particularly informative and captures the extensive research being done in those areas.

All in all, the book gives a very timely review of these varied topics, with up-to-date references. The illustrations provided by the chapter authors are top-notch and very helpful to the understanding of the topics. Although not every reader may wish to go through every page of this reference text, this reviewer, for one, will be going back section by section many times in the years to come. The concise setting of the assembled knowledge and the insight that the authors give on each of these complex subjects are quite valuable in promoting understanding of the topics described and their potential medical importance.

*N. Rebecca Haley, MD*

**Vice President, StemCo Biomedical, Inc.**

2810 Meridian Parkway, Suite 148

Durham, NC 27707
Monoclonal antibodies available at no cost. The Laboratory of Immunochemistry at the New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for screening for antigen-negative donors and for typing patients’ RBCs with a positive DAT. Monoclonal antibodies available include anti-M, -Fya, -K, -Kpa, -Jsβ, -Doβ, -Wrβ, and -Rh17. For a complete list of available monoclonal antibodies, please see our Web site at www.nybloodcenter.org/framesets/FS-4C7.htm. Most of those antibodies are murine IgG and, thus, require the use of anti-mouse IgG for detection, i.e., anti-K, -k, and -Kpa. Some are directly agglutinating (anti-M, -Wrβ, and -Rh17), and a few have been humanized into the IgM isoform and are directly agglutinating (anti-Jsβ and -Fya). The monoclonal antibodies are available at no charge to anyone who requests them. Contact: Marion Reid (mreid@nybloodcenter.org) or Gregory Halverson (ghalverson@nybloodcenter.org), New York Blood Center, 310 East 67th Street, New York, NY 10021.

New York State Blood Bank Association annual meeting. The Blood Banks Association of New York State, Inc., will hold its 53rd annual meeting June 9–11, 2004, in Albany, NY. The preliminary program includes bacterial contamination, stem cells, quality improvement, cost containment in the transfusion service setting, technical workshops, an infectious disease update, and a nursing track. Advance registration is encouraged. For more information and registration, contact: Kevin Pelletier, MT(ASCP), Blood Banks Association of New York State, Inc., PO Box 38002, Albany, NY 12203-8002; phone: (518) 485-5341; e-mail: BTRAXESS@health.state.ny.us.

Annual Symposium. The National Institutes of Health, Department of Transfusion Medicine, will hold their 24th annual symposium, Immunohematology and Blood Transfusion, on September 23, 2004. The symposium is co-hosted by the Greater Chesapeake and Potomac Region of the American Red Cross and is free of charge. Advance registration is encouraged. For more information and registration, contact: Karen Byrne, NIH/CC/DTM, Bldg. 10/Rm. 1C711, 10 Center Drive, MSC 1184, Bethesda, MD 20892-1184; e-mail: kbyrne@mail.cc.nih.gov; or visit our Web site: www.cc.nih.gov/dtm>education.

HEMATOLOGÍA HABANA’ 2005–First Announcement. The 5th National Congress and the 7th Latin American Meeting in Hematology, Immunology, and Transfusion Medicine will present a scientific program at the International Conference Center, Havana, Cuba, May 16–20, 2005. A preliminary program lists malignant hemopathies, disorders of RBC membranes, immunotherapy, histocompatibility, immunohematology, hemolytic disease of the newborn, and blood components as some of the topics. For more information contact: Prof. José M. Ballester, President, Organizing Committee, Hematology Habana’ 2005, Apartado 8070, Ciudad de la Habana, CP 10800, Cuba.

Workshop on Blood Group Genotyping. The ISBT/ICSH Expert Panel in Molecular Biology has recommended that a workshop be held on blood group genotyping by molecular techniques. The results would culminate in a report at the ISBT Congress, July 11–15, 2004, in Edinburgh, Scotland. It was decided that only laboratories that provide a reference service in blood group genotyping would be included in the workshop. One of the aims of the workshop would be to establish an external quality assurance plan. If you have any suggestions as to how the workshop should be organized, we would be grateful for your opinions. If you are interested in taking part in such a workshop, please contact Geoff Daniels (geoff.daniels@nhs.uk). Offer presented by Geoff Daniels, Martin L. Olsson, and Ellen van der Schoot.
Masters (MSc) in Transfusion and Transplantation Sciences

At

The University of Bristol, England

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2004 and will last for one year. A part-time option lasting three years is also available. There may also be opportunities to continue studies for PhD or MD following MSc. The syllabus is organised jointly by The Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Transplantation Sciences. It includes:

• Scientific principles underlying transfusion and transplantation
• Clinical applications of these principles
• Practical techniques in transfusion and transplantation
• Principles of study design and biostatistics
• An original research project

Applications can also be made for Diploma in Transfusion and Transplantation Science or a Certificate in Transfusion and Transplantation Science.

The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site:

http://www.bloodnet.nbs.nhs.uk/ibgrl/MSc/MScHome.htm

For further details and application forms please contact:

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Southmead Hospital, Westbury-on-Trym, Bristol BS10 5NB, England
FAX +44 1179 595 342, TELEPHONE +44 1779 595 455, E-MAIL: ben.bradley@bristol.ac.uk
The Department of Transfusion Medicine, National Institutes of Health, is accepting applications for its 1-year Specialist in Blood Bank Technology Program. Students are federal employees who work 32 hours per week. This program introduces students to all areas of transfusion medicine, including reference serology, cell processing, and HLA and infectious disease testing. Students also design and conduct a research project. NIH is an Equal Opportunity Employer. Application deadline is June 30, 2004, for the January 2005 class. Contact: Karen M. Byrne, NIH/CC/DTM, Bldg. 10/Rm. 1C711, 10 Center Drive, MSC 1184, Bethesda, MD 20892-1184; phone: (301) 496-8335; or e-mail: kbyrne@mail.cc.nih.gov
NATIONAL REFERENCE LABORATORY FOR BLOOD GROUP SEROLOGY

### Immunohematology Reference Laboratory
AABB, ARC, New York State, and CLIA licensed
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(215) 451-2538—Fax

**American Rare Donor Program**
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(215) 451-2538—Fax
ardp@usa.redcross.org

**Immunohematology**
(215) 451-4902—Phone, business hours
(215) 451-2538—Fax
immuno@usa.redcross.org

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- Solid phase red cell adherence (SPRCA) assay
- Monoclonal antibody immobilization of platelet antigens (MAIPA)

For information, e-mail: immuno@usa.redcross.org or call:

Maryann Keashen-Schnell
(215) 451-4041 office
(215) 451-4205 laboratory

Sandra Nance
(215) 451-4362

Scott Murphy, MD
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Answers to Crossword Puzzle Number 4

Reprinted as published in the Red Cell Free Press 1978;3(No. 3)1.
SCIENTIFIC ARTICLES, REVIEWS, AND CASE REPORTS

Before submitting a manuscript, consult current issues of Immunohematology for style. Type the manuscript on white bond paper (8.5” × 11”) and double-space throughout. Number the pages consecutively in the upper right-hand corner, beginning with the title page. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 6 under Preparation
8. Figures—see 7 under Preparation

Preparation of manuscripts

1. Title page
   A. Full title of manuscript with only first letter of first word capitalized (bold title)
   B. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES and J.H. BROWN
   C. Running title of ≤ 40 characters, including spaces
   D. 3 to 10 key words
2. Abstract
   A. One paragraph, no longer than 300 words
   B. Purpose, methods, findings, and conclusions of study
3. Key words—list under abstract
4. Text (serial pages)
   Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.
   A. Introduction
      Purpose and rationale for study, including pertinent background references.
   B. Case Report (if study calls for one)
      Clinical and/or hematologic data and background serology.
   C. Materials and Methods
      Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patients’ names or hospital numbers.
   D. Results
      Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
   E. Discussion
      Implications and limitations of the study links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.
5. Acknowledgments
   Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
6. References
   A. In text, use superscript, arabic numbers.
   B. Number references consecutively in the order they occur in the text.
   C. Use inclusive pages of cited references, e.g., 1431–7.
   D. Refer to current issues of Immunohematology for style.
7. Tables
   A. Head each with a brief title, capitalize first letter of first word (e.g., Table 1. Results of ...), and use no punctuation at the end of the title.
   B. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
   C. Place explanations in footnotes (sequence: *, †, ‡, §, ‡, ††).
8. Figures
   A. Figures can be submitted either by e-mail or as photographs (5” × 7” glossy).
   B. Place caption for a figure on a separate page (e.g., Fig. 1. Results of ...), ending with a period. If figure is submitted as a glossy, place first author’s name and figure number on back of each glossy submitted.
   C. When plotting points on a figure, use the following symbols if possible: ○ ● ▲ ■ △ □ ▢.
9. Author information
   A. List first name, middle initial, last name, highest academic degree, position held, institution and department, and complete address (including zip code) for all authors. List country when applicable.

SCIENTIFIC ARTICLES AND CASE REPORTS SUBMITTED AS LETTERS TO THE EDITOR

Preparation
1. Heading—To the Editor:
2. Under heading—title with first letter capitalized.
3. Text—write in letter format (paragraphs).
4. Author(s)—type flush right; for first author: name, degree, institution, address (including city, state, ZIP code, and country); for other authors: name, degree, institution, city, and state.
5. References—limited to ten.
6. One table and/or figure allowed.

Send all submissions (original and two copies) to:
Mary H. McGinniss, Managing Editor, Immunohematology,
10262 Arizona Circle, Bethesda, MD 20817 and e-mail your manuscript to Marge Manigly at mmanigly@usa.redcross.org
What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:

- Serve as regulatory, technical, procedural and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in blood transfusion medicine
- Conduct research in transfusion medicine

Who are SBBs?

Supervisors of Transfusion Services
Managers of Blood Centers
LIS Coordinators
Educators

Supervisors of Reference Laboratories
Research Scientists
Consumer Safety Officers

Quality Assurance Officers
Technical Representatives
Reference Lab Specialist

Why be an SBB?

Professional growth
Job placement
Job satisfaction
Career advancement

How does one become an SBB?

- Attend a CAAHEP-accredited Specialist in Blood Bank Technology Program OR
- Sit for the examination based on criteria established by ASCP for education and experience

Fact #1: In recent years, the average SBB exam pass rate is only 38%.
Fact #2: In recent years, greater than 73% of people who graduate from CAAHEP-accredited programs pass the SBB exam.

Conclusion:

The BEST route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program

Contact the following programs for more information:

<table>
<thead>
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