Mathematics and transfusion medicine

Roberto Reverberi

This issue of Blood Transfusion contains the first article of a short series on mathematical aspects of transfusion medicine.

In more than three decades of professional activity, I have noticed that there are some topics of transfusion medicine in which a mathematical treatment is important or even essential.

I am not referring to theoretical questions, but to practical applications: in fact, in the course of the series, I hope the reader will appreciate that understanding the mathematical aspects is invaluable as a guide to practical decisions.

With this objective in mind, I have chosen a few topics, such as the kinetics of removal in exchange transfusion and therapeutic apheresis, the factors influencing the antigen-antibody reaction in immunohaematology, the confidence limits of the leucocyte count in leucoreduced blood components.

As my objective is essentially practical, I decided to give readers the possibility to perform the calculations themselves, using common computer programmes, such as Excel and the like.

Therefore, each article is accompanied by instructions on how to programme a spreadsheet so as to obtain the desired results.

Although the instructions are, in most cases, elementary, my experience suggests that they will not be useless: those who are not particularly versed in mathematics or information sciences often get lost, when confronting such problems, and a certain uneasiness about these issues is very common among doctors.

In order not to discourage those readers, the mathematical details are reduced to a minimum or confined to within appendices.

Lastly, this is an open series: interested readers are free to propose new topics or, even better, to send their contributions.

"La filosofia* è scritta in questo grandissimo libro che continuamente ci sta aperto innanzi a gli occhi (io dico l’universo), ma non si può intendere se prima non s’impara a intendere la lingua, e conoscere i caratteri, ne’ quali è scritto. Egli è scritto in lingua matematica …"

("Philosophy* is written in this grand book – I mean the Universe – which stands continually open to our gaze, but it cannot be understood unless one first learns to comprehend the language and interpret the characters in which it is written. It is written in the language of mathematics …")

(Galileo Galilei, Il Saggiatore, 1623)
The genetics of the Rhesus blood group system*

Willy A. Flegel

Institut für Klinische Transfusionsmedizin und Immunogenetik Ulm und Institut für Transfusionsmedizin, Universität Ulm, Germany

The Rhesus factor is clinically the most important protein-based blood group system. With 49 antigens so far described, it is the largest of all 29 blood group systems. The unusually large number of Rhesus antigens is attributable to its complex genetic basis. The antigens are located on two Rhesus proteins - RhD and RhCE - and are produced by differences in their protein sequences. In CD nomenclature, they are termed CD240D and CD240CE. Unlike proteins of other blood groups, Rhesus proteins are expressed only in the membranes of red blood cells and their immediate precursors.

Rhesus is second in its clinical importance only to the ABO blood group. Since the introduction of postpartum anti-D prophylaxis in the late 1960s, and combined pre- and postpartum anti-D prophylaxis in the early 1990s, the incidence of haemolytic disease in newborns due to alloimmunization has been reduced by more than 90%. Up to 1% of all pregnant women have clinically significant anti-erythrocyte antibodies. Anti-D remains the main indication for phototherapy or exchange transfusions in newborns, and pregnant women who are D negative show an above average incidence.

The five most important Rhesus antigens are the cause of most alloimmunizations following blood transfusion. According to the German haemotherapy guidelines, D negative transfusion recipients must always be given D negative erythrocyte products. Since 2000, women of reproductive age and girls have also received transfusions compatible for further Rhesus antigens such as C, c, E and e in addition to the K antigen of the Kell blood group.

This procedure also applies to patients who receive regular transfusions or have immunohaematological problems, like anti-erythrocyte allo- and autoantibodies. In the case of autoantibodies, their exact specificity is not usually determined. Although one thirds of such autoantibodies are directed at Rhesus proteins, this has virtually no practical consequences for treatment.

The D antigen, discovered in 1939, was the first Rhesus antigen to be described. D positive patients were termed Rhesus-positive. In 1946, a quantitative variant with a weakly expressed D antigen was discovered and termed "Du". This variant, now called "weak D", is of clinical and diagnostic importance.

Since 1953, it has been clear that there are also qualitative variants of the D antigen. Although patients with this partial D variant are positive for the D antigen, they can also form anti-D.

The genetic basis

In order to understand the genetic basis of diseases, it is important to understand individual differences in genetic variability, as well as their frequency and distribution in the population. There is usually a close correlation between the genotype and the expressed phenotype. Thus, taking a change in the RHD gene as an example, it is possible to make inferences about the expression of the RhD protein in the erythrocyte membrane. As is the case with many D variants, modified RhD protein can have important implications for transfusion related antigenicity.

The molecular basis of the RH alleles

The first Rhesus gene, the RHCE gene, was discovered in 1990. The RHD gene was found two years later, and the total deletion of this gene ascertained as the cause of the European D negative phenotype.

*Part of this review was presented by the Author during the XXXIX SIMTI Congress (Paestum, SA, 4-7 October, 2006)
More than 170 alleles have been found on the RHD gene since. The site has still not been explored fully, even 15 years after the first RH gene was cloned. DNB, the commonest of all European partial D alleles, was described as recently as 2002.

In 2002, comparisons between the Human Genome Project and the Mammal Genome Project increased understanding of the formation of the two RH genes on chromosome 1 (figure 1).

Most mammals only have one RH gene, whose position corresponds to the human RHCE gene. The RHD gene arose from the duplication of an ancestral RH gene during mammalian evolution. An RH deletion occurred during the evolution of hominids, so that many modern humans completely lack the RHD gene. This haplotype (glossary) is the leading cause of the D negative phenotype worldwide.

The RH alleles can be grouped according to their molecular structure. For the most part, these groups show point mutations (SNP, single nucleotide polymorphisms) which cause missense, nonsense, frame shift or splice site mutations (glossary). RHD-CE-D hybrid alleles are often formed by gene conversion.

The examples of molecular changes and their effects on the D antigen (Table I) show how the D antigen phenotype correlates with the molecular structure.

### The molecular basis of the Rhesus phenotypes

The two Rhesus proteins, RhD and RhCE, are very similar, differing in only 36 of the 417 amino acids, which they each comprise. Each has twelve segments within the erythrocyte membrane and six extracellular loops (figure 2). Both the amino (NH₃) and the carboxyl (COOH) terminal are located within the cell.

### D negative phenotype

The clinically essential difference between Rhesus positive and Rhesus negative hinges on the presence or absence of the D antigen. The molecular basis of this difference is the deletion of the RHD gene, which is the leading cause of D negative phenotype. The examples of molecular changes and their effects on the D antigen (Table I) show how the D antigen phenotype correlates with the molecular structure.

#### Table I - Molecular changes in RHD alleles and their correlation with phenotypes of the D antigen

<table>
<thead>
<tr>
<th>Classification of antigen change</th>
<th>D antigen phenotype</th>
<th>Molecular basis</th>
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<tbody>
<tr>
<td>Partial D</td>
<td>Qualitatively altered</td>
<td>Amino acid substitution on the external surface of protein segment exchange on the outer surface</td>
<td>Missense mutation</td>
<td>RHD(G355S) DNB</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Weak D</td>
<td>Qualitatively altered</td>
<td>Substitution of amino acids in the membrane or extracellular</td>
<td>Missense mutation</td>
<td>RHDV270G</td>
<td>Weak D type 1</td>
<td>Unknown</td>
</tr>
<tr>
<td>DEL</td>
<td>Quantitatively markedly altered</td>
<td>Strongly reduced expression</td>
<td>Missense mutation</td>
<td>RHD/M291I</td>
<td>a/a**</td>
<td>Unknown</td>
</tr>
<tr>
<td>Druglike</td>
<td>Druglike</td>
<td>Absent protein expression</td>
<td>Gene deletion</td>
<td>RHD(C226S)</td>
<td>Druglike a/a**</td>
<td>Impossible</td>
</tr>
<tr>
<td>Antithetical</td>
<td>Presence of antigen</td>
<td>Missense mutation in amino acid position 226 codes for antigen E</td>
<td>Missense mutation</td>
<td>RHCE allele Abs226 codes for antigen E</td>
<td>a/a**</td>
<td>E antigen</td>
</tr>
</tbody>
</table>

* see glossary; ** not assigned

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Duplication of the RH gene and deletion of the RHD gene. The ancestral condition is shown as the RH gene locus in the mouse. The single RH gene is adjacent to the three genes SMP1, P29-associated protein (P) and NPD014 (N). Duplication created a second, reversed RH gene in humans, which is located between N and SMP1. At the insertion points before and after the RHD gene is a DNA segment about 9,000 nucleotides or base pairs (bp) long. The two DNA segments flank the RHD gene and are termed the upstream or downstream Rhesus box. In the RHD positive haplotype, the RHD gene could be lost again through recombination (figure 3). The scale gives the approximate length of 50,000 nucleotides in the genomic DNA.

absence of the RhD protein in the erythrocyte membrane (D positive resp. D negative).

It is unusual for erythrocyte or other cell proteins to be lacking entirely in many humans. This particular genetic feature contributes to the strong antigenicity of the RhD protein. During duplication of the ancestral RH gene, two DNA segments were formed, known as the Rhesus box (Figure 1). The RHD deletion resulted from an unequal crossover (figure 3), which occurs when two DNA segments are highly homologous, such as those of the Rhesus box. The RHD negative haplotype commonest among Europeans is characterized by a hybrid Rhesus box. Subtle molecular differences between the various forms of the Rhesus box are used for genetic testing.

The molecular basis of D antigen variants

Aside from lack of the RhD protein, the D negative phenotype is caused mainly by a series of changes in the RhD protein, which in turn change the phenotype of the D antigen.

Depending on the phenotype and their molecular structure, these RHD alleles are classified as either partial D, weak D or DEL.

Partial D

The RhD protein traverses the erythrocyte membrane several times, leaving only part of the protein exposed at the surface (Figure 2). If an amino acid is substituted in a portion of the RhD protein which is located at the outer surface of the erythrocyte membrane, single epitopes of the D antigen can be lost or new antigens can be formed. DNB is the commonest European partial D (Table I). D categories are a subgroup of partial D. The structure of the RH gene site facilitates gene conversions (figure 4). In the RHD gene some homologous exons of the RHCE gene will be inserted, forming a hybrid Rhesus allele which expresses a corresponding hybrid protein. This is how the D categories III to VI arose. The changes usually affect a long string of amino-acids, which is always located on the erythrocyte surface.

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Figure 2 - The Rhesus protein in the erythrocyte membrane. Both Rhesus proteins show 417 amino acids, shown here as circles. Mature proteins in the membrane lack the first amino acid. The amino acid substitutions which distinguish the RhD from the RhCE protein are shown in yellow, with the four amino acids which code for the C antigen in green and the one which codes for the E antigen in black. The single amino acid substitutions which code for partial D are in blue, those which code for weak D are in red. The mutations, identified by the Ulm group, are in light blue and orange.

Figure 3 - Deletion of the RHD gene. Deletion of the RHD gene resulted from recombination between an upstream and a downstream Rhesus box on two different chromosomes. This is termed an unequal crossover. When the two crossed strands separate (from A over the recombination site to B), the DNA at the RH gene site completely lacks the RHD gene (C). This haplotype (C) occurs in about 41% of the population. An individual homozygous for this haplotype (about 17% are) is D-negative.
If an amino acid substitution is located within the erythrocyte membrane or the cytoplasm, this will result in a weak D phenotype (figure 2)\(^1\). Integration of the RhD protein into the membrane will be hindered, leading to quantitative weakening of the D antigen. There is usually no qualitative change, and hence no anti-D immunization. The weak D type 1 is the commonest in Europe (Table I). A particularly weakly expressed D antigen is termed DEL (earlier Del), because it could only be demonstrated using elution. In elution, antibodies are separated from erythrocytes to demonstrate them in the eluate. The molecular changes are more severe than those seen with weak D, considerably hindering but not completely preventing integration into the cell membrane. All DEL alleles are rare in Europe, but up to 30% of all apparently D negative individuals in East Asia are bearers of the DEL allele RHD(K409K)\(^{10,12}\).

The C/c and E/e antigens

The clinically important Rhesus antigens C, c, E and e are the result of RhCE protein changes at only five amino acid locations (figure 2). Antigens are termed antithetical if a protein can present only one of them. They are caused by protein polymorphisms. Often there are two variants of a protein, which differ at only one amino acid location, such as the Rhesus antigens E and e. RHCE alleles showing the amino acid proline at position 226 express the E antigen, whereas RHCE alleles showing the amino acid alanine at this position express the e antigen (Table I). Similar differences between two RHCE alleles account for the antithetical C and c antigens. The antigen pairs C/c and E/e are not antithetical, however, because they result from substitutions at different locations. The four possible combinations occur at different frequencies (among Europeans: Ce > ce > cE > CE) and are inherited as haplotypes.

Clinical applications

Genetic investigation, like all investigations in medicine, should only be carried out in the context of a clear aim\(^{13}\). As far as transfusions are concerned, molecular biological techniques already are being used to provide cost-effective answers to a number of clinically important questions. Methods used include polymerase chain reaction (PCR) for gene amplification and subsequent identification by electrophoresis, nucleotide sequencing and hybridization on biochips.\(^{14}\)

Anti-D in patients

The clinical problems encountered are caused by a small number of RHD alleles. Patients usually show partial D, in some rare cases weak D immunized by normal D antigen. Since category VI (DVI) is the most important of these, the Authors recommend using monoclonal anti-D antibodies for typing, which do not react with DVI\(^{15,16}\). 

Figure 4 - Category DVI as a result of gene conversion. The two RH genes lie on their chromosome pointing in opposite directions (i.e., a cluster). When the chromosome folds, the two RH genes are adjacent, now pointing in the same direction. This configuration allows gene conversion to occur, whereby a DNA segment is transferred from one gene to another. The middle section of the RHD gene (yellow) is substituted by the corresponding homologous section of the RHCE gene (green) (A). This type of gene conversion is responsible for the RHD-CE(DVI-6) D allele, which codes for the D category VI of the molecular type 3 (DVI type 3) (B). Exons 1 to 10 are drawn on both RH genes (C). Due to the contrary directions, the terminal exons of the two RH genes (RHD and RHCE exon 10) lie closest to each other. On the RHD gene, exons 3 to 6 are substituted by the homologous exons of the RHCE gene.
Genetics of Rhesus system

This procedure was included in the German haemotherapy guidelines in 1996 and has not been changed since. DVI carriers are therefore deliberately typed as false negatives to prevent transfusions with D positive blood and likely anti-D immunization[20].

A foetus can be shown to be D positive by demonstrating foetal DNA in the plasma of peripheral maternal blood. Anti-D prophylaxis is unnecessary if the foetus is D-negative. This could save about 40% of all the anti-D prophylaxis currently given during pregnancy. This method was developed in countries bordering on Germany, where intensive efforts are under way to implement this approach to genetic diagnosis[19].

Prenatal diagnosis

If the foetus needs to be tested for D antigen, amniocentesis or sampling from the trophoblast is the method of choice[26]. Cordocentesis is no longer performed. As already mentioned, maternal plasma may be able to be used in future.

Having a child and anti-D antibodies

If the father is heterozygous for the RHD deletion, there is a 50% chance of the foetus being D-negative, in which case the pregnancy essentially free of any haematological risk. If the father is homozygous for the RHD gene, the foetus will definitely inherit the D antigen, which could influence the couple’s decision on whether to have a child or not.

For several decades, it was impossible to determine whether an individual is heterozygous or homozygous for RHD because serological methods are unsuitable. With the advent of the genetic diagnosis of the hybrid Rhesus box, however, the possibilities have been expanded considerably: If the father is D-positive, it is now sufficient to test him for the RHD deletion.

Use in other diseases

If the standard serological methods fail, genetic diagnosis is the method of choice for a reliable blood group typing of patients after a transfusion and those with auto- or alloimmune haematological anaemias. Although transfused leucocytes can under certain circumstances persist for years, they will not interfere with routine genetic diagnosis.

Blood donors

Appropriate investigation for the RHD gene can identify apparently D negative donors, who in reality are weak D or DEL, thus ensuring that their blood will be given only to D positive recipients[20]. Without genetic diagnostics, D-negative blood transfusion recipients will continue to be immunized by the D antigen contained in such blood[24-27]. Donors who so far were misidentified as being D negative and whose erythrocytes are D-Ds chimeras can now be identified correctly. Lifelong chimerism can result from monochorionic twin pregnancies. Any transfusion from donor sources such as these can result in anti-D immunization, because they also contain several millilitres of erythrocytes with a perfectly normal D positive phenotype. This D positive blood can only be detected using genetic investigation, not with routine serological methods[25,27]. Any case of anti-D immunization is of considerable clinical importance for girls and women of reproductive age. In the case of a D positive pregnancy,
this would be likely to result in Rhesus haemolytic disease of the newborn.

The function of Rhesus proteins

Most blood group proteins have a known function. While purifying human Rhesus proteins, American physician Peter Agre discovered a water transporter protein. This discovery earned him the 2003 Nobel Prize for Chemistry. Despite intensive efforts, however, no function has been found for the RhD and RhCE proteins. Although the Rhesus associated antigen (RhAG), a Rhesus homologue contained in erythrocytes, can transport ammonium ions, the Rhesus proteins themselves could not be shown to have any such function. One possible function under investigation involves the exchange of CO₂ and even O₂. Other information on the RH alleles will only be gained from the everyday clinical application of genetic diagnostics, which could thus contribute to identifying their function.

From the perspective of basic research, where transfusion medicine will continue to make a contribution, scientific work on Rhesus and other blood groups has been quite productive, and is anything but finished.

Outlook

Genetic diagnosis has been used for blood group typing in clinical transfusion medicine ever since 2000. As antenatal care has shown, genetic blood group typing has led to a better quality of care, by helping to avoid potential side effects and reducing costs. This is a rare combination, and justifies the extra costs involved in optimizing care via the use of genetic diagnostic techniques. As well as improving patient care, these methods can fuel the development of new methods, which will also be used for health care outside of Germany. European departments of transfusion medicine are leading the field in molecular blood group diagnostics and applications, and will continue to contribute to improving patient care.

Common Genome Variability Terms

SNP (single nucleotide polymorphism)
Point mutation. Variability in a nucleotide sequence due to change of a single nucleotide.

Allele
The expression of a coding or non coding nucleotide sequence (the exon resp. intron of a gene) with two or more variants, often differing by only a point mutation.

Genotype
A pair of alleles or variants of a nucleotide sequence occurring at homologous sites on paired chromosomes.

Haplotype
A combination of alleles or variants of a nucleotide sequence located close together on the same chromosome, and usually inherited together.

Missense mutation
Amino acid substitution in a protein caused by a point mutation. It can alter the function or antigenicity of a protein.

Nonsense mutation
A stop codon caused by a point mutation which prematurely stops synthesis of the amino acid chain, leading to loss of protein function of its expression.

Silent mutation
A point mutation which does not change the amino acid at the site. Although the protein is unchanged, it still can be associated with a clinically relevant phenotype and be used diagnostically.

Frame shift mutation
The loss or insertion of one or two nucleotides which shifts the reading frame and premature stops protein synthesis (or extends it in some rare cases), resulting in loss of protein function or expression.

Splice site mutation
A point mutation at a splice site (the exon-intron junction), causing faulty splicing of messenger RNA (mRNA) and skipping an exon, thus changing the amino acid sequence. Leads to loss of protein function or expression.

Gene conversion
The non-reciprocal exchange between two or more homologous genes, whereby a certain nucleotide sequence on a gene is substituted by a sequence on another gene, which is located on the same chromosome (conversion in cis).

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Key Word: Rhesus, blood group, molecular diagnostic, transfusion, pregnancy.

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Genetics of Rh system


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Correspondence: Prof. Dr. Willy A. Flegel
Institut für Transfusionsmedizin
Universitätsklinikum Ulm
Helmholtzstrasse 10 - 89081 Ulm - Germany
E-mail: willy.flegel@uni-ulm.de

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New technologies in immunohaematology

Fernanda Morelatti1, Wilma Barcellini2, Maria Cristina Manera, Cinzia Paccapelo1, Nicoletta Revelli1, Maria Antonietta Villa1, Maurizio Marconi1

1 Centro Trasfusionale e di Immunoodiagnosi, Dipartimento di Medicina Rigenerativa, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Istituto di Ricovero e Cura a Carattere Scientifico, Milano, Italy
2 U.O. Ematologia 2, Dipartimento di Medicina e Medicina Speciale, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Istituto di Ricovero e Cura a Carattere Scientifico, Milano, Italy

Introduction

Since the discovery of the ABO system, numerous important innovations have contributed to a continuous, rapid evolution in the diagnostic methods for in vitro measurements of the antigen-antibody reaction, allowing a significant improvement in the compatibility between blood from donors and the recipients. Apart from the introduction of ABO typing, these methods include the determination of Rh type and phenotype, the direct and indirect antiglobulin tests, cross-matching and consequent identification of antigens and antibodies of clinical relevance, the use of low ionic strength additives and enzyme treatments, the development of monoclonal reagents and solid-phase and microcolumn platforms for performing the pre-transfusion tests.

Since transfusion safety depends on a series of strictly inter-related processes1, among which pre-transfusion tests have a predominant role, in recent years some of the new technologies that integrate the classical techniques in immunohaematology have become valid instruments for improving the safety of transfusions. The aim of this review is to illustrate the principles and practical applications of these emerging techniques used in our laboratory to identify antigens and antibodies, in cases of red cell or platelet immunisation.

Automation for complex cases

The most recent data in the literature2 indicate that, still nowadays, incorrect identification of samples and errors in performing tests are the most frequent causes of transfusion reactions and complications, with sometimes dramatic consequences3 for health.

The use of completely automated systems, indivisible from the use of information technology, is the most efficient strategy for achieving two main goals in the field of immunohaematology:

- reducing transfusion risks related to human errors, by automating the stages related to identifying samples, selecting reagents, performing and interpreting results and transferring data to the laboratory’s information management system;
- guaranteeing the traceability of all the elements involved in the analytic process, which can be archived and remain accessible after the test has been performed.

Following the 1990s the use of automated systems increased in all industrialised countries in parallel with the development and marketing of new technologies; these systems have increased the objectivity and stability of the results and the standardisation of the process with respect to the traditional liquid phase methods.

The most widely used systems are based on the use of:

- microcolumns, with different types of commercial products, which enable the results to be seen after the passage of red blood cells through a matrix containing the reagents; the main advantage of this technology, which has led to its widespread use, is mainly related to the fact that the antiglobulin test can be carried out without washing steps;
- polystyrene microplates with wells pre-coated with lyophilised red bloods or platelets, or anti-erythrocyte or anti-platelet antibodies: the antibodies present are revealed by immuno-adherence after addition of red blood cells coated with an anti-IgG human antiglobulin; a more recent system, based on the use of microplates sensitised by an anti-IgG human antiglobulin, enables the reaction to be visualised through magnetised red cells and for the antiglobulin test to be carried out without washing steps.

The combined use of these techniques and latest generation, completely automated instruments has enabled automation of even more sophisticated immunohaematology tests. These tests can be used in
particular conditions to resolve the most complex cases. In our Centre, full automation has been efficiently applied in the following conditions.

1) **Large-scale cell phenotyping**
A fully automated high output system based on solid-phase technology is currently used for the red cell extended phenotype.

The system enables typing of 14 red blood cell antigens of the greatest transfusional relevance, using samples of blood in anticoagulated (EDTA) blood, processed within 3-6 days of collection, and a combination of:
- polyclonal antisera (anti-Fy, anti-Fy, anti-Jka, anti-Jkb, anti-S, anti-s, anti-C, anti-c, anti-Jk, anti-Lu, anti-Kp, anti-Vel and anti-PP1Pk); related to the peculiarity of the reactions evaluated in a pilot study
- Identification of red blood cell antibodies.

a. A manual method had to be used in order to identify the specificity of the antibodies themselves. In 233 (0.17%) of these cases no inconclusive results were observed with anti-Fy, 
- b. plasma from immunised donors (anti-Ge2, anti-PP1Pk, anti-U, anti-Vel), diluted 1:5 in saline and stored at +4°C until use.

The instrument processes samples in batches of 50-100, dispensing 12 samples, 7 typing reagents and 1 negative control/sample for each plate.

Over a period of 12 months, this procedure was used to carry out 134,129 typings on 12,644 blood donors attending the 'Rare Blood Group Bank – Reference Centre, Region of Lombardy'. In 1% of the cases (1,339 typings) the result was not conclusive (indeterminate/doubtful/invalid) at the first test.

The commercial antisera were the cause of inconclusive results in 156 (0.12%) typings and human plasma in 1,183 (0.9%) typings.

No inconclusive results were observed with anti-Fy, anti-K, and anti-k specificities. A high percentage of repetitions were required after the first test with the anti-Vel and anti-PP1Pk plasma samples (803 tests for anti-Vel, 163 for anti-PP1Pk), related to the peculiarity of the reactions of the antibodies themselves. In 233 (0.17%) of these cases a manual method had to be used in order to identify the antigens.

2) **Identification of red blood cell antibodies**

The possibility of automating this complex process was evaluated in a pilot study carried out in 2004 using a completely automated instrument based on standard commercial panels and microcolumn technology.

One of the most important difficulties in the identification of the red cell antibodies was related to the antigen profile of the commercial panels, which was scarcely useful when mixtures of antibodies were present.

In these cases, further extensively typed red blood cells are necessary to achieve complete identification of the specificities involved.

Two new solid-phase panels, selected for Rh phenotype and also prepared for use in a completely automated instrument, recently became available. These were evaluated for their performance in an automated process when mixtures of red cell antibodies are present, that include also Rh specificities.

Two 14-cell panels were used for this evaluation: the first panel consisted of homozygous cells for the C and E antigens and the second comprised 13 cells negative for the Rh(D) antigen and a control, Rh(D) positive cell.

The panels were used to test samples from 61 non-immunised subjects and 104 immunised subjects, who had undergone complete immunohaematological investigations, prior to the evaluation.

Among the subjects investigated, 75 had single antibody (28 anti-D, 7 anti-C, 2 anti-CDE, 26 anti-E, 1 anti-c, 1 anti-C, 6 anti-c, 1 anti-K, 2 anti-Jc, and 1 anti-M) while the other 29 patients had mixtures of antibodies (4 anti-D, 20 anti-E, 3 anti-C, 8 anti-c, 5 anti-Ce, 11 anti-K, 1 anti-Kp, 3 anti-Jka, 1 anti-Jkb, 2 anti-Fya, 1 anti-Fyb, and 5 anti-S). The negative samples were evaluated with the two panels and the positive samples with one of the panels according to the known specificity (57 with the first panel and 47 with the second). The tests were carried out using the method defined by the instrument, which required interpretation of the results by the operator. In the case of discrepancy with the previous result, the specificity involved was verified with the manual methods used in the laboratory. Complete correspondence with known results (Table I) was observed in non-immunised subjects and in 91 (87.5%) of the 104 samples with red cell antibodies. In 11 of these, 12 additional antibodies were found, of which 9 were identified with the first panel (2 anti-c, 1 anti-E, 1 anti-Jc, 1 anti-K, 1 anti-Cw, 1 anti-Kpa and 2 autoantibodies reacting only in the solid-phase) and three with the second panel (2 autoantibodies against low incidence antigens and 1 autoantibody reacting only in the solid-phase).

Two class IgM antibodies were not detected by the first panel (1 anti-K) and by the second panel (1 anti-M).

3) **Selecting platelet concentrates for patients with immunological refractoriness**

The condition known as ‘immunological refractoriness’...
to transfusion of standard platelet concentrates is one of the most important complications in subjects requiring transfusion support and indicates repeated, poor increments in post-transfusion platelet count (three consecutive platelet counts below 5 x 10^9/L). This condition, which can be associated with severe clinical complications, is caused by the presence of class I antibodies against human leucocyte antigens (HLA) and is observed in 13-14% of patients with leukemia transfused with standard blood components and in 3-4% of subjects transfused with leucocyte-depleted blood components. The traditional transfusion approach is based on choosing HLA identical or compatible donors or on selecting appropriate donors through tests of platelet compatibility. In our laboratory we have chosen the latter approach by using the platelet concentrates (from buffy-coats or obtained through apheresis products) present in the daily inventory of the units and a completely automated instrument based on the solid-phase technology.

Over a period of 33 months, post-transfusion platelet count increments were evaluated in 40 refractory subjects (27 women, 13 men) transfused with platelet concentrates selected using this procedure \(^{(11,12)}\) and the increments were related to known detrimental clinical factors and to the post-transfusion increments (at 1 hour after the transfusion), observed after the last three transfusions with standard platelet concentrates (not selected by platelet cross-match). Within 48 hours from starting the selection procedure, the subjects under consideration had been transfused with 569 platelet concentrates (median value 8 concentrates/patient, containing 202 ± 71 x 10^9 platelets/patient), obtained from buffy-coats or by apheresis procedures. The median pre-transfusion platelet count was 7.7 ± 5.5 x 10^9/L, and the post-transfusion platelet increments exceeded 10,000 platelets/µL in 68% of the cases (Figure 1).

The post-transfusion counts in subjects with detrimental factors were lower (28.9 ± 20.3 x 10^9 platelets/µL at 1 hour after the end of the transfusion) than those observed in subjects without such factors (35.9 ± 21.2 x 10^9 platelets/µL at 1 hour after the end of the transfusion).

### Table I - Results of the identification of red cell antibodies carried out with a completely automated instrument

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Complete agreement (n.)</th>
<th>Additional antibodies detected by the instrument (n.)</th>
<th>Antibodies not detected by the instrument (n.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunised subjects</td>
<td>91</td>
<td>11 samples</td>
<td>2 IgM antibodies (anti-K, anti-M)</td>
</tr>
<tr>
<td>Non-immunised subjects</td>
<td>61</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(*) 2 anti-E, 1 anti-C, 2 anti-BC, 1 anti-K, 1 anti-C, 1 anti-N, 3 autoantibodies, 2 antibodies against low incidence antigens.

Among the many red cell immunohaematology problems, one of the most difficult to manage is autoimmune haemolytic disease with a negative direct antiglobulin test (DAT). In order to resolve the diagnostic problem in these cases, a battery of investigations must be used, carried out with different methods (agglutination tests, solid phase tests, ELISA, flow cytometry, immunoradiometric tests, evaluation of complement consumption). One particularly useful test in the study of these complications is the mitogen stimulation test (MS-DAT), designed by Barcellini\(^{(13,14)}\) and colleagues, which is used to evaluate the prevalence of positive results in subjects with autoimmune haemolytic anaemia (AIHA) in clinical remission or in an active phase of the disease. The MS-DAT test is carried out by stimulating whole blood cultures from the investigated subjects with mitogen (phytohaemagglutinin – PHA; phorbol-12-myristate-13-acetate – PMA; or pokeweed mitogen-PWM), the production of antibodies in the culture after stimulation is evaluated by a competitive ELISA in solid phase. An agglutination DAT, using the standard test-tube method, a DAT with red cells washed in low ionic strength solution or cold physiological saline, and a solid phase test for immunoadherence were carried out in parallel in all subjects \(^{(4-6)}\). Using this technique, 33 subjects with AIHA were studied (of whom 27 in an active phase of disease with a positive DAT, and 6 subjects with previous AIHA who had become DAT negative) and 7 subjects with DAT-negative AIHA, whose disease had been diagnosed on the basis of exclusion of all other causes of haemolysis and on the response to steroid therapy. Furthermore, we studied 69 subjects with chronic B-cell lymphocytic leukaemia (B-CLL), a disease associated with a high prevalence of autoimmunity against red blood cells, with blood transfusions.
or without a positive DAT. Mitogenic stimulation caused an increase in the quantity of IgG that adhered to red cells in culture in patients with AIHA and B-CLL, but not in controls (Table II). The MS-DAT was positive in 6 patients with previous AIHA who had become DAT-negative (Table III, subjects 1-6) and in 7 subjects with DAT-negative haemolytic disease was diagnosed as having an autoimmune nature by exclusion (Table III, subjects 7-13). Finally, MS-DAT was positive in one third of the subjects affected by B-CLL without signs of haemolysis. The MS-DAT was also carried out during clinical monitoring of patients with AIHA, with results strictly related to the clinical course of the disease, to the changes in haemolytic parameters and to the response to therapy (Figure 2).

### Table II - The effect of mitogenic stimulation in the presence of antibodies against autologous red blood cells in whole blood culture

<table>
<thead>
<tr>
<th></th>
<th>Not stimulated</th>
<th>PHA ($)</th>
<th>PMA ($)</th>
<th>PWM ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with AIHA</td>
<td>322±49*</td>
<td>623±122**</td>
<td>465±55***</td>
<td>635±134**</td>
</tr>
<tr>
<td>Patients with B-CLL</td>
<td>134±15**</td>
<td>207±29*</td>
<td>182±37**</td>
<td>183±25*</td>
</tr>
<tr>
<td>Controls</td>
<td>75±7</td>
<td>75±9</td>
<td>70±6</td>
<td>76±14</td>
</tr>
</tbody>
</table>

(Phytohaemagglutinin; ($) Phorbol-12-myristate-13-acetate; (k) Pokeweed mitogen; * p<0.001; ** p<0.01; *** p<0.05)

The values (mean ± SD) are expressed as IgG ng/mL [33 patients with autoimmune haemolytic anaemia (AIHA), 69 with B-cell chronic lymphatic leukaemia (B-CLL) and 81 controls]

### Molecular techniques

The advances made over the last 20 years in defining the molecular nature of red cell and platelet antigens has enabled the use of molecular techniques in various fields of immunohaematology, particularly in some situations in which the traditional methods are difficult or impossible to apply. The most relevant of these include:

- determination of the antigen profile in subjects:
  - recently transfused or with a positive DAT,
  - with suspected variants, weak expression or null phenotype of antigens or with discrepancies in blood group determination that cannot be resolved with the traditional serological techniques;
- antenatal investigations to evaluate:
  - transfusion with cross-match negative platelet concentrates
  - transfusion with cross-match negative red blood cell concentrates

Figure 2 - MS-DAT in the clinical follow-up of AIHA. The levels of haemoglobin (●), haptoglobin (●) and MS-DAT (●) are expressed as percentages of the values at the start of the follow-up. For the first patient (figure on the left) these values were: Hb=5.8 g/dL, haptoglobin=200 mg/L, MS-DAT=4,121 ng/mL IgG. For the second patient (figure on the right) they were: Hb=13.6 g/dL, haptoglobin=590 mg/L, MS-DAT=41 ng/mL IgG.

Table III - Clinical and laboratory characteristics of patients with DAT-negative and MS-DAT positive AIHA

<table>
<thead>
<tr>
<th>N.</th>
<th>Sex</th>
<th>Hb g/dL</th>
<th>Total bilirubin (indirect) µmol/L</th>
<th>Reticulocytes %</th>
<th>Haptoglobin mg/L</th>
<th>LDH U/L</th>
<th>Steroid therapy</th>
<th>DAT (*)</th>
<th>MS-DAT IgG ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>13.3</td>
<td>8 (5)</td>
<td>1.4</td>
<td>1,200</td>
<td>420</td>
<td>Yes</td>
<td>neg</td>
<td>279</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>12.4</td>
<td>15.7 (12)</td>
<td>0.8</td>
<td>2,290</td>
<td>286</td>
<td>Yes</td>
<td>neg</td>
<td>521</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>12.3</td>
<td>10.5 (7)</td>
<td>0.7</td>
<td>1,830</td>
<td>377</td>
<td>No</td>
<td>neg</td>
<td>433</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>13.1</td>
<td>10 (9)</td>
<td>1.8</td>
<td>1,200</td>
<td>472</td>
<td>No</td>
<td>neg</td>
<td>302</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>12</td>
<td>10 (8)</td>
<td>0.9</td>
<td>690</td>
<td>303</td>
<td>Yes</td>
<td>neg</td>
<td>256</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>15</td>
<td>13.7 (8)</td>
<td>0.2</td>
<td>870</td>
<td>291</td>
<td>No</td>
<td>neg</td>
<td>322</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>12</td>
<td>27 (22)</td>
<td>3.7</td>
<td>200</td>
<td>480</td>
<td>No</td>
<td>neg</td>
<td>813</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>10.9</td>
<td>28 (22)</td>
<td>4.4</td>
<td>200</td>
<td>420</td>
<td>No</td>
<td>neg</td>
<td>433</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>11.1</td>
<td>20 (18)</td>
<td>1.1</td>
<td>200</td>
<td>550</td>
<td>No</td>
<td>neg</td>
<td>1,230</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>5.3</td>
<td>20 (19)</td>
<td>6.9</td>
<td>200</td>
<td>232</td>
<td>No</td>
<td>neg</td>
<td>1,860</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>8.8</td>
<td>n.d.</td>
<td>4.3</td>
<td>200</td>
<td>520</td>
<td>Yes</td>
<td>neg</td>
<td>516</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>10.8</td>
<td>40 (27)</td>
<td>15.9</td>
<td>200</td>
<td>470</td>
<td>No</td>
<td>neg</td>
<td>856</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>11.1</td>
<td>9 (8)</td>
<td>0.9</td>
<td>500</td>
<td>338</td>
<td>No</td>
<td>neg</td>
<td>314</td>
</tr>
</tbody>
</table>

Normal range

Women 11.5-15
Men 14-16.5

(<2%) 600-3,000 250-460

(*) The DAT was carried out by agglutination, using the standard test-tube method, with washed red blood cells in low ionic strength solution or cold physiological saline, and in solid phase by immunoadherence.

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New immunohaematological technologies

- the risk of haemolytic disease of the newborn,
- the risk of neonatal alloimmune thrombocytopenia,
- RHD zygosity;
c) large scale typing of blood donors for red cell and platelet antigens, when typing antisera are not available or difficult to obtain.

Molecular characterisation of antigens is essential in transfused immunised patients, in order to select compatible units of red blood cells and in pregnant women, in order to decide whether to administer RhoD prophylaxis.

Over the course of about 2 years, molecular techniques were used to study 28 blood donors (5 suspected ABO variants, 5 discrepancies in the typing of other red cell antigens and 15 donors negative for high incidence antigens) and 40 patients sent for immunohaematological investigations (6 with ABO discrepancies and 4 with Rh discrepancies in the agglutination techniques, 11 transfused subjects for bone marrow transplantation or thalassaemia or malignancy, 10 DAT-positive transfused subjects, 3 immunised pregnant women and their partners, whose offspring were suspected to be affected by foetal or neonatal alloimmune disease, 4 subjects with suspected haemolytic thrombocytopenia, 1 foetus with suspected haemolytic disease, 4 subjects with haemolytic transfusion reactions and 1 with platelet-specific alloimmunisation). Molecular typing was carried out using DNA, at a concentration of 5- and 1 with platelet-specific alloimmunisation). Molecular typing was carried out using DNA, at a concentration of 5-10 ng, extracted from peripheral blood in EDTA, using the salting out method. The commercial kits used were based on the Polymerase Chain Reaction Sequence-Specific Primers (PCR-SSP) method, and prepared according to a carefully defined process including all the phases between the different steps involved in the relevant field. The findings were 3 ABO variants (A\(\beta\), 4D variants (1 DFR, 1 Rh33, 1 Dweak type 1 and 1 Dweak type 5), 14 cases of absence of a high incidence antigen (3k, 2La\(a\), 1 Co\(a\), 7Fy\(b\), 1 HPA-1a), in another 6 cases (5 donors and 1 patient) genomic typing revealed the presence of antigens that the serological techniques had not detected (3 Fy\(b\)weak, 2La\(a\) and 1 antigen of the KEL system).

A second study was carried out to identify donors of platelets negative for human platelet antigens (HPA). The anti-HPA alloantibodies and relative antigens were involved in cases of post-transfusion purpura, in immunological refractoriness to standard platelet transfusions and in cases of allo-immune spontaneous thrombocytopenia. The availability of donors of known platelet type is essential in order to ensure effective transfusions of platelet concentrates in these subjects. However, it is difficult to identify such subjects because of the scarcity of specific typing antisera to use in the classical methods (in ELISA, in flow cytometry or in solid phase). Genomic DNA from 149 Caucasian, group O blood donors was analysed in order to determine the genotype of the HPA-1a, HPA-1b, HPA-2a, HPA-2b, HPA-3a, HPA-3b, HPA-5a and HPA-5b antigens. The PCR-SSP method and a commercial kit were used. The allelic, phenotypic and genotypic frequencies observed in this population of blood donors were comparable with those reported in other European studies (Table IV).

Conclusions

In this article we have presented our recent experience with some of the new technologies internationally applied to the field of red cell and platelet immunohaematology to reduce the risk of incompatibility between donors and recipients and, therefore, to improve transfusion safety.

The use of automation, in particular, seems to be a valid approach for reducing the risk related to human error and guaranteeing the traceability of all the operative phases in all critical processes involving large numbers, such as extended red cell typing, identification of antibodies in multiply immunised subjects and in the transfusion therapy of patients affected by immunological refractoriness to standard platelet concentrates.

The use of molecular techniques has become indispensable, in combination with agglutination methods, for defining the red cell antigen profile in recently transfused and immunised subjects or in cases in which discrepancies cannot be resolved with traditional serological techniques and in the consequent selection of compatible red blood cell units. These techniques represent the only method available for characterising the platelet antigen profile, which cannot be determined otherwise because of the lack of suitable typing reagents, and in antenatal investigations to evaluate the risk of haemolytic disease of the newborn, the risk of alloimmune thrombocytopenia and the antigen profile of the foetus. The mitogen stimulation test is a clinically important assay in the management of cases of suspected AIHA, that gives negative results in the traditional test.

Finally, it should be emphasised that transfusion safety depends on a series of processes which must be improved and monitored over time and that the use of new technologies is only one element in the transfusion procedure.

It is, therefore, essential to use new technologies within a carefully defined process including all the phases between selection of the donor, the transfusion of blood components and the follow-up of the patients.

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Table IV - Results of HPA typing and a comparison with frequencies reported in other European countries

<table>
<thead>
<tr>
<th>HPA antigen</th>
<th>Observed (1)</th>
<th>Italy (Fratellanza 2005)</th>
<th>the Netherlands (Simsek 1993)</th>
<th>Finland (Kokonaki 1990)</th>
<th>Wales (Sellers 1999)</th>
<th>Germany (Kold 1993)</th>
<th>Austria (Holensteiner 1995)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1a</td>
<td>0.832</td>
<td>0.838</td>
<td>0.86</td>
<td>0.825</td>
<td>0.834</td>
<td>0.852</td>
<td></td>
</tr>
<tr>
<td>HPA-1b</td>
<td>0.167</td>
<td>0.162</td>
<td>0.154</td>
<td>0.14</td>
<td>0.173</td>
<td>0.168</td>
<td></td>
</tr>
<tr>
<td>HPA-2a</td>
<td>0.879</td>
<td>0.85</td>
<td>0.934</td>
<td>0.91</td>
<td>0.902</td>
<td>0.940</td>
<td>0.918</td>
</tr>
<tr>
<td>HPA-2b</td>
<td>0.12</td>
<td>0.15</td>
<td>0.066</td>
<td>0.09</td>
<td>0.094</td>
<td>0.060</td>
<td>0.082</td>
</tr>
<tr>
<td>HPA-3a</td>
<td>0.701</td>
<td>0.658</td>
<td>0.555</td>
<td>0.59</td>
<td>0.607</td>
<td>0.616</td>
<td>0.612</td>
</tr>
<tr>
<td>HPA-3b</td>
<td>0.298</td>
<td>0.342</td>
<td>0.445</td>
<td>0.41</td>
<td>0.393</td>
<td>0.584</td>
<td>0.388</td>
</tr>
<tr>
<td>HPA-5a</td>
<td>0.859</td>
<td>0.920</td>
<td>0.902</td>
<td>0.95</td>
<td>0.903</td>
<td>0.889</td>
<td>0.892</td>
</tr>
<tr>
<td>HPA-5b</td>
<td>0.14</td>
<td>0.081</td>
<td>0.088</td>
<td>0.05</td>
<td>0.097</td>
<td>0.111</td>
<td>0.108</td>
</tr>
</tbody>
</table>

(1) Centro Trasfusionale e di Immunomutologia, Dipartimento di Medicina Rigenerativa, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Istituto di Ricovero e Cura a Carattere Scientifico, Milano (Italy)
New immunohaematological technologies

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The first data from the haemovigilance system in Italy

Adele Giampaolo, Vanessa Piccinini, Liviana Catalano, Francesca Abbonizio, Hamisa Jane Hassan

Reparto di Metodologie Transfusionali, Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Roma, Italy

Introduction

The clinical risk of transfusions is perceived predominantly as the risk of acquiring infectious diseases. In reality, over the last 20 years, the incidence of transfusion-related transmission of diseases has decreased significantly, thanks to greater attention given to the stages of selecting donors and screening the units collected. The real transfusion process, mostly carried out in hospital wards and operating theatres, tends to be less considered, but now needs to be monitored to increase the safety of the whole process. Errors related to the identification of the patient, of the sample test-tube and of the blood component expose patients to risk and, in some cases, increase the risk of mortality.

From the monitoring of the adverse reactions due to transfusions, reported by countries in which a haemovigilance system has been active for some time,
it can be deduced that immunological adverse events, transfusion-related acute lung injury (TRALI) and errors in the transfusion process are much more likely than infections transmitted by transfusion of blood components9,10.

The ultimate purpose of haemovigilance, defined as the surveillance of unexpected or adverse reactions in donors and recipients and as epidemiological surveillance in donors, is to prevent the repetition of adverse events and reactions9. In fact, the information obtained from haemovigilance systems can contribute to improving the safety of blood collection and transfusion by: a) supplying the medical community with a valid source of information about the risks related to transfusion; b) indicating corrective measures to prevent the repetition of some accidents or dysfunctions of the transfusion process, including particularly significant ones, such as samples taken from the wrong person, mistaken identification of the sample, errors in the request form, and blood transfused to the wrong person; c) alerting the hospital wards and Transfusion Structures (TS) about adverse events that could involve several patients, such as those related to the transmission of infectious diseases and to the collection and processing of the blood.

In Europe, the first haemovigilance systems were activated in France in 19944 and in the United Kingdom in 19965, although these two systems differ greatly. The former is obligatory and requires notification of adverse events of all degrees of severity; notification of near miss errors (that is, errors recognised before the transfusion) is voluntary. The latter system is voluntary and collects information on severe adverse reactions, transfusion errors and near miss errors. Since Directive 2002/98/EC came into force, the introduction of haemovigilance systems has become a priority for all countries in the European Community.

In Italy, the surveillance of adverse events in recipients was activated at the end of 2004 by the National Institute of Health [Istituto Superiore di Sanità - ISS]8. There were already systems for monitoring adverse reactions in some Regions6; at a national level, efforts were made to guarantee the homogeneity of the data collected by using the same forms. The proposed form for national surveillance was designed by Transfusion Medicine specialists and agreed upon through consultatory meetings with representatives of the Regional Centres of Coordination and Compensation [Centri Regionali di Coordinamento e Compensazione – CRCC]. Subsequently, dedicated software was developed, based on the paper form. This software, called PETRA (Programma degli Errori Transfusionali e delle Reazioni Avverse – Programme for Transfusion Errors and Adverse Reactions), was distributed by the ISS to all TS. Participation in the haemovigilance system was not obligatory, but was strongly recommended by the institutions (ISS and CRCC).

The assimilation of Directive 2002/98/EC of the European Parliament and Council of Europe, during 2005, has made the notification of severe unwanted reactions and incidents obligatory: "Whatever severe incident, whether due to an accident or an error, related to the collection, control, processing, storage, distribution or assignment of blood or blood components, which could influence their quality and safety, as well as any severe unwanted reaction observed during or after a transfusion which could be related to the quality and safety of the blood and its components, or to human error, is notified to the region or autonomous province involved, which, in its turn, notifies the ISS" (article 13 in4).

The Italian system of haemovigilance is substantially in line with the European Directive, although it lacks the surveillance of adverse or unexpected events in donors and registration at a national level of severe incidents related to the collection, processing and storage of blood and blood components, which could have effects on the quality and safety of the blood component. In the future, changes should be made to the current national haemovigilance system in order to render it conform with the new requisites. Epidemiological surveillance of donors was started at a national level in 1989 for HIV and in 1999 for HBV, HCV and Treponema pallidum. This monitoring enables estimations of regional and national prevalences and incidences of the major transfusion-transmissible infections and also enables evaluation of the residual risk of such infections8,10.

The collection and analysis of data on undesired effects of transfusion rely on a close collaboration between the TS, which supply the blood components, and the hospital wards. This collaboration is essential, in order to ensure complete investigations of every unfavourable event. The Committee for the Good Use of Blood, by involving all the professional figures
“dealing with blood”, could represent the context in which to spread the culture of haemovigilance, making collaboration between TS and hospital wards more possible.

**Methods**

The collection of haemovigilance data is the responsibility of the 326 Italian TS distributed throughout the country and located within hospitals. Inside the TS, blood is donated, tested and processed to produce the blood components, that are then sent to the wards; the TS must then receive, from the doctor who uses the transfusion therapy, documentation on every transfusion and any adverse reactions (article 15, paragraph 3

The TS store and manage the information, filling in the computerised PETRA form for every case identified. The records are periodically sent to the CRCC, which then transmit the regional data to the ISS. The data collected can be used at local, regional, national and international levels.

The haemovigilance system calls for the registration of immediate transfusion reactions (haemolysis, TRALI, bacterial contamination, anaphylactic shock, etc.), late effects (haemolysis, graft-versus-host disease (GvHD), post-transfusional purpura, etc.) and transfusion of wrong blood components.

The system is also designed to collect information on near miss errors, that is, mistakes recognised before the transfusion, which could have led to the transfusion of a mistaken blood group or the registration, collection or management of a wrong, inappropriate or unusable blood component.

The information required by the PETRA notification form are those considered minimum by Recommendation R(95)15: (a) date of birth, sex and identification code of the patient transfused; (b) number of the units and identification codes of the blood components involved in the adverse event; (c) description of the type of blood component, its method of preparation and the conditions and duration of storage of the blood component prior to its transfusion; (d) severity of the event, reported according to a graded scale (mild symptoms, long-term morbidity, immediate threat to life, death); (e) imputability, that is, the relation between the unfavourable effects observed and the blood component transfused, using a graded scale.

The PETRA forms sent by the Region of Piemonte are filled in electronically, interfacing the PETRA software with that of the "Form for recording adverse events of transfusion therapy", used by the Region.

The incidence of adverse events was calculated based on the number of units of blood components distributed, which is monitored by the National and Regional Register of Blood and Plasma.

**Results**

**Survey of adverse events in 2005**

In 2005, the percentage of the 326 Italian TS that participated in the haemovigilance survey was 38.4%, which was almost double that in 2004 (21.0%). This percentage also includes the TS that did not use PETRA and those that stated the absence of adverse reactions.

Table I reports the percentage participation in 2004-2005. The regions in which all the TS participated in 2005 survey were Friuli-Venezia Giulia, Liguria, Lombardia, the Marche, Piemonte, the Autonomous Province of Bolzano and Valle d’Aosta; the coverage in military TS was 100%. The rate of responses was high in Lazio and Veneto.

Considering the number of units of blood components distributed in 2005 by the TS, which were included in survey (1,834,474), the system monitored 49.6% of the units distributed in Italy (3,701,724) (Figure 1).

In 2005, there were reports of 1,495 adverse reactions, 823 of which were reported using a summary data-sheet other than PETRA, such that, in most cases of events notified, a description of the causative role of the transfusion in the adverse reaction was not given.

Overall, 0.8 reactions were reported every 1,000 units of blood components distributed.

Almost all the adverse events reported were acute: 46.9% of the reactions were febrile type reactions and 38.7% were of an allergic nature (urticaria and anaphylactic reactions). Only six delayed type adverse reactions were reported: four late haemolytic reactions, one case of GvHD and one HIV infection (Figure 2).

The collection of data carried out with different methods by the structures that participated in the haemovigilance system in 2005 was not homogenous and did not enable further analysis.
Table I - Participating Transfusion Structures

<table>
<thead>
<tr>
<th>Region</th>
<th>2004 (%)</th>
<th>2005 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abruzzo</td>
<td>7.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Basilicata</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Calabria</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Campania</td>
<td>0.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>0.0</td>
<td>25.1</td>
</tr>
<tr>
<td>Friuli Venezia Gorizia</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Lazio</td>
<td>14.7</td>
<td>58.3</td>
</tr>
<tr>
<td>Liguria</td>
<td>90.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Lombardia</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Marche</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Molise</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Piemonte</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Puglia</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Sardegna</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Sicilia</td>
<td>36.4</td>
<td>53.3</td>
</tr>
<tr>
<td>Toscana</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Autonomous Province of Bolzano</td>
<td>25.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Autonomous Province of Trento</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Umbria</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Valle d’Aosta</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Veneto</td>
<td>31.6</td>
<td>63.2</td>
</tr>
<tr>
<td>Military structures</td>
<td>0.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 1 - Monitoring of the blood components distributed –2005
Overall, 986 forms were returned (Table II), of which 871 reported adverse reactions and 63 reported near miss errors or errors; 52 forms could not be evaluated (Figure 3A).

In 848 of the forms, the adverse reaction was attributed to the transfusion; in 65% of these (n=555) the association with the transfusion was stated to be strong (certain or probable cause); only these were taken into consideration for the analysis (Figure 3B).

As far as regards the severity of the reactions, these caused mild symptoms in 65.8% of the reported cases and long-term morbidity in 31.9%, were life-threatening in 2.1%, and led to death in 0.2%. Figure 4 shows the severity of the reactions in the events in which the transfusion was probably or certainly the cause of the reaction. The only death was due to an acute haemolytic reaction caused by ABO incompatibility. Life-threatening events were anaphylactic reactions (n=5), volume overload syndrome (n=2), TRALI (n=1), febrile reaction (n=1), urticarial reaction (n=1) and reactions defined as "other" (n=2).

Almost all the adverse reactions reported were acute; only three were delayed (late haemolysis). As regards the type of reaction, 46.4% were of an allergic nature (urticaria and anaphylactic reactions) and 33.9% of a febrile type (Figure 5).

The type of blood component involved in the adverse reactions was reported in 92.9% of the cases

### Table II - PETRA forms received

<table>
<thead>
<tr>
<th>Region</th>
<th>Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abruzzo</td>
<td>6</td>
</tr>
<tr>
<td>Campania</td>
<td>4</td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>65</td>
</tr>
<tr>
<td>Friuli Venezia Giulia</td>
<td>13</td>
</tr>
<tr>
<td>Lazio</td>
<td>25</td>
</tr>
<tr>
<td>Liguria</td>
<td>18</td>
</tr>
<tr>
<td>Marche</td>
<td>95</td>
</tr>
<tr>
<td>Piemonte</td>
<td>483*</td>
</tr>
<tr>
<td>Puglia</td>
<td>8</td>
</tr>
<tr>
<td>Sardegna</td>
<td>4</td>
</tr>
<tr>
<td>Sicilia</td>
<td>44</td>
</tr>
<tr>
<td>Autonomous Province of Bolzano</td>
<td>1</td>
</tr>
<tr>
<td>Veneto</td>
<td>287</td>
</tr>
<tr>
<td>Military structures</td>
<td>1</td>
</tr>
</tbody>
</table>

(*) See "Methods" section

**PETRA 2004-2005**

**Adverse reactions**

Overall, 986 forms were returned (Table II), of which 871 reported adverse reactions and 63 reported near miss errors or errors; 52 forms could not be evaluated (Figure 3A).

In 848 of the forms, the adverse reaction was attributed to the transfusion; in 65% of these (n=555) the association with the transfusion was stated to be strong (certain or probable cause); only these were taken into consideration for the analysis (Figure 3B).

As far as regards the severity of the reactions, these caused mild symptoms in 65.8% of the reported cases and long-term morbidity in 31.9%, were life-threatening in 2.1%, and led to death in 0.2%. Figure 4 shows the severity of the reactions in the events in which the transfusion was probably or certainly the cause of the reaction. The only death was due to an acute haemolytic reaction caused by ABO incompatibility. Life-threatening events were anaphylactic reactions (n=5), volume overload syndrome (n=2), TRALI (n=1), febrile reaction (n=1), urticarial reaction (n=1) and reactions defined as "other" (n=2).

Almost all the adverse reactions reported were acute; only three were delayed (late haemolysis). As regards the type of reaction, 46.4% were of an allergic nature (urticaria and anaphylactic reactions) and 33.9% of a febrile type (Figure 5).

The type of blood component involved in the adverse reactions was reported in 92.9% of the cases.
(n=516), in which the transfusion was stated to be probably or certainly the cause of the reaction: red blood cells in 374 reactions, platelets in 73, fresh-frozen plasma in 53, whole blood in 5 and stem cells in 11.

**Errors**

There were 16 notifications of errors, concerning transfusions given to the wrong patient: the transfusions were ABO incompatible in 56% of the cases, Rh incompatible in 6% and not specified in the other 38% of the cases. The cases notified were errors of identification of the patient and/or sample test-tube: in one case, the sample used for the request was taken from a different patient; in another case, the error was caused by the patient having the same name as another patient, already registered in the TS archives; in one other case, the transfusion request, related to another patient, was not checked; in the other cases, the error was caused by not correctly identifying the patient in
the ward at the time of the transfusion. In the six reports in which the patients’ outcome was described, one patient died, three had no consequences and two had mild symptoms.

**Near miss errors**

There were 47 reports of near miss errors, that is, errors recognised before the transfusion. Six of the forms did not report the type of error that had occurred, the other forms described, for the most part (75%), errors occurring in the wards at the time of taking the samples, errors in filling in the identifying data on the test-tube, and errors in completing the request form.

The other 25% of the reports concerned errors occurring in the TS at the time of accepting the request and the samples, when issuing the units, and when conducting serological tests in the laboratory. Table III presents the various types of near miss errors in detail.

**Discussion and conclusions**

This study, the first Italian report on the notification of adverse reactions to transfusions, refers to years 2004 and 2005 and concerns about 50% of all blood components distributed in the nation.

In this first analysis of national haemovigilance data it was considered useful to analyse both the PETRA forms that were received and the summary data-sheets (concerning only the type of adverse reaction) supplied by those regions, which, in compliance with Italian legislation, had already set up a system for recording transfusions and notifying adverse reactions. These different systems of reporting did, however, lead to a lack of homogeneity in the notifications, thus enabling only a partial analysis of the events notified. A more detailed analysis was possible only of the PETRA forms.

The notification form was designed by transfusion medicine specialist and agreed upon consultatory meetings with representatives of CRCC and other components of the transfusion system. Health care managers were informed of the new haemovigilance system by the ISS and invited, as presidents of the Committees on the Good Use of Blood, to spread the culture of haemovigilance and to encourage wards, in which blood products are used, to notify an adverse events occurring after a transfusion.

In 2005, 1,495 adverse reactions were notified, including those reported by TS not using PETRA. The TS participating, including those which declared no transfusion reactions, had distributed 1,834,474 units of blood components. Therefore, the reported rate of reactions was 0.8/1,000 units. This is undoubtedly an underestimate, given that the French system has consistently recorded about 3 adverse reactions/1,000 units of blood components since 1998.

The percentage of notifications varied greatly from region to region. The scarcity of notifications (under-reporting) is a problem in all haemovigilance systems and can have various causes: the adverse reaction and/or its relation with the transfusion is not always recognised; when it is recognised, the importance of notifying it is not accepted as a tool to improve transfusion safety; staff may be afraid of disciplinary action in the case of notified errors.

Almost all the adverse events notified were acute reactions, since only five delayed reactions were notified. An almost complete lack of recording of late reactions (delayed haemolytic reactions, GvHD, post-transfusional purpura, viral, bacterial and parasitic reactions)...

<table>
<thead>
<tr>
<th>Table III - Description of near miss errors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the ward</strong></td>
</tr>
<tr>
<td>Sample taken from wrong person</td>
</tr>
<tr>
<td>Errors in data identifying the test-tube</td>
</tr>
<tr>
<td>Error in request form</td>
</tr>
<tr>
<td>Incongruities between data on request form and on test-tube</td>
</tr>
<tr>
<td><strong>In the Transfusion Structure</strong></td>
</tr>
<tr>
<td>Exchange of samples</td>
</tr>
<tr>
<td>Error during the stage of accepting the request in the management system</td>
</tr>
<tr>
<td>Mistaking one patient for another</td>
</tr>
<tr>
<td>Error in serological test in the laboratory</td>
</tr>
<tr>
<td>Issue of wrong units</td>
</tr>
<tr>
<td>Predeposited unit belonging to another patient sent to another TS</td>
</tr>
</tbody>
</table>
infections, haemochromatosis) was reported. In France, these reactions represent 35% of all the adverse events reported¹.

The lack of agreed definitions negatively affects data collection. For example, it would be useful to have shared diagnostic criteria for TRALI which is a transfusion reaction that is difficult to diagnose, because the symptoms can be very different and there is not a specific diagnostic test. TRALI is included as an adverse reaction in the United Kingdom haemovigilance system (Serious Hazards of Transfusion (SHOT)) which, between 1996 and 2004, reported 162 cases, of which 36 were fatal; of these, 13 were considered probably or certainly due to the transfusion. In France, this adverse reaction was not diagnosed until 2002 (although it was, perhaps, sometimes reported as volume overload). After an effort of the haemovigilance system to improve the diagnosis of this reaction, TRALI is now better identified and studied. In 2003, 15 cases probably or certainly due to transfusions were identified, of which three were fatal. Only one case of TRALI was reported in the Italian system, leading to the suspicion that there is underreporting of this reaction, perhaps due in part to the difficulty in its diagnosis.

The types of transfusion reactions were febrile in 46.9% of the cases and allergic (urticaria and anaphylactic reactions) in 38.7%. The analysis of only those cases, in which the transfusion was certainly or probably causal, notified with the PETRA system, showed opposite proportions of these two pathologies: 46.5% of the reactions were of an allergic nature and about 34% were febrile-type reactions. These percentages are in line with data reported by other haemovigilance systems.

The promotion of a different culture, in which notifying errors is in some way fostered, is a fundamental step in any attempt to identify and tackle system defects in the transfusion chain. Errors and near miss errors (the latter being more frequent) represent failures in the system and by analysing them, critical points to be kept under control can be identified.

It has emerged from the reports on haemovigilance in France and the United Kingdom that transfusional errors leading to ABO-incompatible transfusions require great attention. Up to 2002, the French haemovigilance system only recorded ABO incompatibilities that had a clinical effect; the notification of clinical grade 0 errors was introduced only subsequently, as an instrument to achieve improvements, even in the absence of adverse reactions.

In Italy, as in other countries, a transfusion reaction due to ABO incompatibility is considered a sentinel event, which can and must be prevented.

"Recommendations for the prevention of transfusion reactions due to ABO incompatibility" has been issued by the Ministry of Health (Office III – Quality of activities and services – General Management of Health Care Planning, levels of care and system ethical principles).

The regions of Veneto and Emilia Romagna were particularly active in notifying errors and near miss errors. Previous implementation of systems for clinical risk management and a culture of reporting adverse events, with the aim of improving patient safety, increased participation to haemovigilance and enable better and greater integration of monitoring systems. Overall, there were nine reported cases of ABO incompatibility, of which one was fatal; all these cases were due to an error in one of the critical steps of identifying the patient. These nine cases account for 0.6% of the events notified.

SHOT found that the overall number of errors was equivalent to 70% of the events notified and that cases of ABO incompatibility accounted for 10% of the events notified¹. The need to educate and train staff is considered fundamental for the function of a haemovigilance system. The data on errors should be monitored for longer, in order that awareness of the importance of notifying errors, by staff, can make the haemovigilance system fully effective.

Estimating the incidence of errors is essential to monitor blood safety and to help health care managers to make informed decisions on systematically introducing instruments for the identification of patients/units of blood components. These instruments are commercially available and already used in some hospitals.

Compared to the organization of the transfusion system in other countries, in which blood banks are completely separate from hospitals, Italian TS are located within hospitals leading to a different management of blood units, presumably with a more effective traceability.

Having overcome the problem of under-reporting, given that the incidence of errors is lower than that...
reported by other countries, it could be concluded that the organisation of the Italian transfusion system is better able to guarantee the correct identification of patients/units of blood components.

Acknowledgements

We thank the CRCC that participated in the national haemovigilance system and, in particular, all the Transfusion Structures that used the PETRA software.

We also thank Dr. Roberto Baroni for his precious contribution in standardising and analysing the clinical data.

References

14) Council of Europe. Committee of Ministers. Recommendation N. R(95) 15. Preparation, use and guarantee of the quality of blood components. Adopted by the Committee of Ministers on 12 October 1995 at the 545th Meeting (issued 10 June 1996)
Tuscan Study on the Appropriateness of Fresh-frozen Plasma Transfusion (TuSAPlaT)

Giancarlo Maria Liumbruno1, Maria Laura Sodini1, Giuliano Grazzini2

1 Servizio di Immuinoneutologa and Medicina Trasfusionale, AUSL N. 6 – Livorno, Italy
2 CRCC Regione Tuscany, Italy

Introduction. The considerable increase in the consumption of fresh-frozen plasma (FFP) recorded in 2002 in the Region of Tuscany made it necessary to check the appropriateness of the use of this blood component in all transfusion facilities in the Tuscan network.

Materials and methods. From July 1, 2003 to December 31, 2005, the Regional Blood Transfusion Co-ordinating Centre carried out an audit on the clinical use of FFP in the 40 structures included in the Tuscan transfusion network. The study had two complementary parts: a review of guidelines on the use of FFP and the involvement of Hospital Transfusion Committees in evaluating the outcome of the audit and in the consequent local policy decisions and educating clinicians.

Results. The data from all 40 of the regional transfusion structures were analysed. The audit, which was initially retrospective, gradually became prospective. The percentage clinical use of FFP decreased, compared to 2002, in each of the 3 years of the study: a) 2003: - 8.92%; b) 2004: - 2.11%; c) 2005: - 1.97%. The inappropriate requests for plasma decreased from 27% to 22.7% of the total. It was possible to classify the inappropriate requests for plasma on the basis of homogeneous, regionally defined criteria. The most frequent inappropriate indication (60.7% of the total) was the use of plasma in the case of haemorrhage in patients with a normal PT and/or PTT or unavailable results. Each hospital revised its own guidelines between 2004 and 2005 and the Hospital Transfusion Committees set up appropriate educational and behavioural interventions.

Conclusions. The capacity of transfusion facilities to make data on the use of blood components available systematically and continuously is an essential feature of clinical governance; systematic clinical auditing increases the level of appropriate behaviours in the transfusion sector, contemporaneously contributing to self-sufficiency in transfusion products, and may direct research towards those clinical settings at greatest risk of inappropriate use of transfusion therapy with FFP.

Key words: fresh-frozen plasma, transfusion, audit, appropriateness.
In Italy, in the 5 years from 2001-2005, the clinical use of fresh-frozen plasma (FFP) has tended to increase, despite 2% reductions in 2002 and in 2005 compared to the consumption in the respective preceding years, 2001 and 2004. In fact, the consumption of FFP increased from 146,844 L in 2001 to 153,493 L in 2005.10-16

In 2002, the Tuscany Regional Blood Transfusion Coordinating Centre (RBTCC) for transfusion services found that the use of FFP in the Region clearly contrasted with the national trend; indeed, that year, the use of FFP reached its peak of 10,625 L (equivalent to about 3 L/1,000 residents/year), with an increase of 4% with respect to 2001. Therefore, in 2003, the RBTCC, in the context of a regional benchmarking project lasting several years, that was aimed at continuous improvement of quality and self-sufficiency of the Tuscan transfusion system, introduced a systematic audit of the appropriateness of the clinical use of FFP in the hospitals making up the regional transfusion network.

Inappropriate transfusion therapy with FFP is probably one of the main avoidable risks for patients and it is known that guidelines are not, per se, able to influence clinical practice13-15, because without the help of other instruments, such as auditing and educational interventions, they may not be able to reach a level of diffusion and acceptance such as to be able to direct clinical practice towards the recommended behaviours.

There is, however, evidence showing that clinical auditing is an educational and behavioural strategy that is effective in altering the level of inappropriate transfusion treatment and is able to contribute to self-sufficiency in blood products, to a reduction in health care costs and to the delivery of better health care.16

The aim of this study was to verify the impact of an auditing process on the appropriateness of the clinical use of FFP and to classify the main causes of the inappropriate use of this product within the regional transfusion network.

Materials and methods

The transfusion system of the Region of Tuscany is spread throughout the region and comprises 15 Services of Immunohaematology and Transfusion Medicine (SIMT) and 25 hospitals, the Tuscan transfusion network is incorporated in regional hospitals, managed by 12 Health Care Companies and by 4 University Hospital Companies, which deliver health care services to a population of about 3,600,000 people.

The study was co-ordinated by the RBTCC and carried out over a period of 3 years, that is, the second semesters of 2003 and 2004 and throughout 2005, in this period all the regional transfusion structures audited the requests received for FFP and, at the same time, undertook two complementary actions: updating their guidelines and involving the Hospital Transfusion Committees (Comitati Ospedalieri per il Buon Uso del Sangue, COUBUS) in the evaluation of the outcome of the audit and in the consequent implications for local policy and education of clinicians.

The audit was conducted by manual review of transfusion requests by medical staff of the SIMT and the Transfusion Sections. Each transfusion structure was asked for the following data for each year: a) method of carrying out the appropriateness audit (retrospective or prospective) of the FFP transfusion requests received; b) total number of requests for FFP received; c) number of FFP requests audited; d) classification of the requests, on the basis of literature data and specific hospital guidelines, into: 1) appropriate; 2) inappropriate and, from 2004, 3) into a grey zone, with the aim of dividing the definitely inappropriate requests from those that were potentially inappropriate; e) classification of the inappropriate requests, from July 2004, according to the following, predefined, homogeneous regional criteria: 1) haemorrhage in a surgical context with PT and/or PTT not available; 2) haemorrhage in a non-surgical context with PT and/or PTT not available; 3) haemorrhage with PT and/or PTT normal; 4) prophylaxis of haemorrhagic events with PT and/or PTT normal or not performed; 5) prophylaxis of haemorrhagic events in the presence of altered PT and/or PTT; 6) hypoalbuminaemia and/or nutritional purposes; 7) requests based on predefined formulas; 8) consumption of more than one of the preceding causes of inappropriate; 9) other causes.

The data collected by the SIMT and the Transfusion Sections were subsequently sent at regular intervals to the RBTCC, which processed them, and reported them together with information on the regional production and consumption of FFP from 2002 to 2005; furthermore, the clinical use of FFP from 2002 to 2005 was also compared with the consumption of red blood cells (RBCs), a parameter used by the RBTCC as a regional indicator of the intensity of health care activities.

The statistical analysis (Student’s t test) was carried out with GraphPad InStat software (version 3.05, GraphPad Software, San Diego, CA-USA).

Results

Data were received from all 40 regional transfusion structures and analysed. Each hospital revised its own

The indications, for which the clinical use of FFP is considered appropriate, are reported below:
- correction of a congenital deficiency of a clotting factor for which the specific factor concentrate is lacking;
- acquired deficiency of multiple clotting factors, when PT and aPTT are > 1.5 normal levels, in the presence of an ongoing haemorrhage or severe risk of haemorrhage;
- use as a fluid replacement in the apheresic treatment of thrombotic microangiopathies;
- reconstitution of whole blood for exchange transfusions;
- hereditary angioedema due to C1 esterase deficiency, in the absence of a specific plasma-derivative.

In the same period, the COBUS of all the structures involved in the study evaluated the results of the audits carried out and involved clinicians in a shared review of the guidelines on transfusion therapy with FFP and in their diffusion within the hospitals.

The overall data for the 3 years examined on the way the auditing was carried out showed that, on average, 56% of the transfusion requests underwent systematic, prospective auditing.

The mean percentage of prospective audits of the requests increased in a statistically significant manner from 30% in 2003 to 65% in 2004 (p = 0.0001) and to 72% in 2005 (p < 0.0001); the increase between 2004 and 2005 was not statistically significant (p = 0.26) (Table I).

The total percentage of requests subjected to appropriateness auditing increased from 39.2% in 2003, to 99.1% in 2005; that of the inappropriate requests changed from 27% in 2003 to 26.7% in 2004, and to 22.7% in 2005; furthermore, in the period of 18 months between July 2004 and December 2005, 5.6% of the requests checked (900/15,996) were classified in the grey zone (Table II).

In the 24 months of the study, the Tuscan transfusion structures received a total of 24,918 requests for FFP and audited 19,070 (76.5%) of them; 13,506 (70.8%) of the requests were considered appropriate, 4,664 (24.5%) inappropriate and 900 (4.7% of the total in the 3 years) fell in the grey zone.

The analysis of the percentage of requests for FFP, which were audited by the individual transfusion structures (data not shown), revealed increases that were statistically significant for the comparison between 2003 and 2004 (p = 0.03) and between 2003 and 2005 (p = 0.02), but not for the comparison between 2004 and 2005 (p = 0.2).

The trends in the percentages of appropriate, not appropriate and grey zone requests, analysed for the individual hospitals for the period under consideration, did not show statistically significant changes (data not shown), although there was a general tendency to a reduction in the total percentage of inappropriate and grey zone requests for FFP.

Compared with the level of use in 2002, the clinical use of FFP (in litres) decreased in each of the 3 years of the study: a) 2003: - 8.92%; b) 2004: - 2.11%; c) 2005: - 1.97%; similarly, there was a reduction in the litres of FFP derived from separation that were used clinically: a) 2003: -13.99%, b) 2004: - 10.13%, c) 2005: - 17.91%, and a steady increase in the percentage of FFP from apheresis used clinically (Table III) (Figure 1).

Table I - Region of Tuscany: trend in the percentage of prospective audits of Fresh Frozen Plasma (FFP) requests from 2003 to 2005

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>30</td>
<td>65</td>
<td>72</td>
<td>56</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>16</td>
<td>36</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>Median</td>
<td>37</td>
<td>77</td>
<td>85</td>
<td>45</td>
</tr>
<tr>
<td>Range</td>
<td>0-45</td>
<td>0-100</td>
<td>0-100</td>
<td>0-100</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>20.3 – 39.7</td>
<td>43.2 – 86.6</td>
<td>52.2 – 91.7</td>
<td>45 – 87</td>
</tr>
<tr>
<td>p 2003 vs 2004</td>
<td>0.0001</td>
<td>&lt; 0.0001</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>2003 vs 2005</td>
<td>0.0001</td>
<td>&lt; 0.0001</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>2004 vs 2005</td>
<td>0.0001</td>
<td>&lt; 0.0001</td>
<td>0.28</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table II - Region of Tuscany: classification of the transfusion requests for fresh-frozen plasma (FFP) received and audited from 2003 to 2005

<table>
<thead>
<tr>
<th>FFP requests</th>
<th>2003 (6 months)</th>
<th>2004 (6 months)</th>
<th>2005 (12 months)</th>
<th>2004-2005 (18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received</td>
<td>7,841</td>
<td>5,978</td>
<td>11,099</td>
<td>17,077</td>
</tr>
<tr>
<td>Audited</td>
<td>3,074</td>
<td>4,997</td>
<td>10,999</td>
<td>15,996</td>
</tr>
<tr>
<td>Audited %</td>
<td>39.2</td>
<td>83.5</td>
<td>99.1</td>
<td>93.7</td>
</tr>
<tr>
<td>Appropriate</td>
<td>2,244</td>
<td>3,263</td>
<td>7,999</td>
<td>11,262</td>
</tr>
<tr>
<td>Appropriate %</td>
<td>73</td>
<td>65.3</td>
<td>72.7</td>
<td>70.4</td>
</tr>
<tr>
<td>Not appropriate</td>
<td>830</td>
<td>1,335</td>
<td>2,499</td>
<td>3,834</td>
</tr>
<tr>
<td>Not appropriate %</td>
<td>27</td>
<td>26.7</td>
<td>22.7</td>
<td>24</td>
</tr>
<tr>
<td>Grey zone</td>
<td>0</td>
<td>399</td>
<td>501</td>
<td>900</td>
</tr>
<tr>
<td>Grey zone %</td>
<td>0</td>
<td>8</td>
<td>4.6</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Figure 1 – Region of Tuscany: fresh-frozen plasma (FFP) from apheresis and from separation used for clinical purposes from 2002 to 2005. The values are expressed in litres and as a percentage of the total.
The year in which the clinical use of FFP was lowest was 2003; there were then moderate increases in 2004 and 2005, although the levels of 2002 were not reached. The increased use of FFP (in litres) from 2003 to 2005 was, nevertheless, percentage-wise, less than that expected on the basis of the increased consumption of RBCs in the same period. RBC use is an indicator of the intensity of health care services recorded by the RBTCC throughout the region; in fact, the consumption of RBC units was 145,498 units in 2002, decreased in 2003 to 144,921 units (-0.39 %) then rose in 2004 to 151,947 units (+ 4.43 %) and in 2005 to 154,080 units (+ 5.98 %) (Figure 2).

The regional production of plasma changed from 57,400 L in 2002 to 65,979 L in 2005 (Table III) (Figure 3). The relationship (in litres) between FFP used clinically and the total amount of FFP produced showed a tendency to decrease with respect to that in 2002: a) 2002: 18.51%; b) 2003: 16.39%; c) 2004: 16.6%; and d) 2005: 15.78%.

In order to classify the types of inappropriate requests according to predefined criteria established by the Region, an analysis was conducted on 15,996 requests (93.7% of the total 17,077 received) for FFP during 18 consecutive months from July 1, 2004 to December 31, 2005; 3,834 (24%) of the requests were considered inappropriate, 11,262 (70.4%) appropriate and 900 (5.6%) fell in the grey zone (Table II).

Table IV reports the types of inappropriate requests and their proportional incidence in decreasing order. The analysis of the types of inappropriate requests recorded by the individual hospitals (data not shown) revealed the following: (i) there were 2,328 inappropriate requests for FFP (60.7% of all inappropriate requests) for the treatment of haemorrhagic events in patients with PT and/or PTT values unavailable or normal: 38.7% of these requests (n=901) came from three University hospitals; (ii) treatment of hypoalbuminaemia and/or nutritional purposes were the reasons for 212 inappropriate requests for FFP (5.5% of the total): 56.6% of these requests (n=120) came from one University hospital.

Discussion
The systematic review of 19,070 requests for FFP, equivalent to 76.5% of all requests for the period 2003-2005, showed that the inappropriate use of FFP in Tuscany decreased from 27% in 2003 to 22.7%, in 2005. The mean reduction over the whole 3-year period was 24.5%; this result, if transferred into daily clinical practice, implies that of every four requests for FFP, one is inappropriate. The percentage of inappropriate clinical use of FFP recorded in this regional study is however, in the lower range (10-74%) reported in previous studies on the same subject carried out between 1986 and 2006, although these were conducted in heterogeneous health care systems, including some non-western countries.6,16-37. The percentage reduction in inappropriate use (4.3%) was detectable, although relatively small; it is compatible with results obtained in other studies carried out in health care systems comparable with the Italian system. In these studies, prospective auditing was combined with corrective actions (such as improving blood component request form) and educational interventions aimed at improving clinical practice.17,29. The decrease in inappropriate requests for FFP during the course of the


Table III - Region of Tuscany: consumption of fresh-frozen plasma (FFP) for clinical use from 2002 to 2005 and production of the FFP in the same period.

<table>
<thead>
<tr>
<th></th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFP produced (L)</td>
<td>57,400</td>
<td>59,056</td>
<td>62,626</td>
<td>65,979</td>
</tr>
<tr>
<td>Difference in production compared to 2002 (%)</td>
<td>+2.85</td>
<td>+9.1</td>
<td>+14.95</td>
<td></td>
</tr>
<tr>
<td>FFP distributed for clinical use (L) from apheresis</td>
<td>5,404</td>
<td>5,186</td>
<td>5,708</td>
<td>6,130</td>
</tr>
<tr>
<td>Difference in the distribution of FFP for clinical use compared to 2002 (%) from apheresis</td>
<td>-4.35</td>
<td>+5.62</td>
<td>+15.43</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10,625</td>
<td>9,677</td>
<td>10,400</td>
<td>10,416</td>
</tr>
</tbody>
</table>
Figure 2 - Region of Tuscany: comparison between the changes in percentage consumption of fresh-frozen plasma (FFP) and red blood cells (RBC) in the years 2003-2005 compared to in 2002.

Figure 3 - Region of Tuscany: production of fresh-frozen plasma (FFP) and clinical use of FFP from 2002 in litres.
study was attributed to various factors, probably acting together: a) the increase in audits of appropriateness (+ 44.3% in 2004 and + 15.6% in 2005) and, in particular, the continuous increase of prospective auditing from 2003, even if this did not reach statistical significance in 2005; b) the interventions carried out between 2004 and 2005, such as the divulgence of the updated guidelines within the hospitals and the education of clinicians promoted by the COBUS. In fact, the COBUS are able to influence the appropriateness of the use of blood components significantly. The results of the present study confirm already existing evidence on the capacity of behavioural interventions to modify the level of inappropriate transfusion therapy. Moreover, this regional study seems to indicate that auditing and educational interventions, particularly if combined with a review of the guidelines on transfusion therapy, can enhance their diffusion and acceptance, improving the appropriateness of blood product use. The analysis of the regional consumption of plasma, during the period of the study, confirmed the increase of appropriateness of FFP therapy. Other indices of the more appropriate use of FFP are the considerable reduction in the clinical use of plasma obtained by separation in favour of that from apheresis (with consequent less exposure of recipients to donors) and the lesser percentage increase in FFP use compared to that of RBC use. The increased use of RBCs in the same period reflects the considerable intensification of health care, that occurred in the Region from 2003 onwards, particularly in transplant medicine. This could explain why, in the same period that prospective auditing increased significantly, there was also a moderate increase in the consumption of FFP, although this never reached the levels of 2002.

The possibility of classifying requests in a grey zone, introduced in the audit from 2004, enabled 900 potentially inappropriate requests to be picked up in the following 18 months. These requests were separated from the definitely inappropriate requests, thus reducing the “bias” of mistaken classification and undoubtedly contributing to reducing the percentage of inappropriate requests.

Furthermore, the use of the grey zone was attributed the capacity to increase the robustness of the studies on the inappropriateness of transfusion therapy, precisely because of the separation that this zone enables between potentially and truly inappropriate transfusions.

The analysis of about 16,000 requests made in 18 consecutive months, showing that 24% of these were inappropriate, enabled the main causes of the inappropriateness to be determined and grouped in predefined classes. The highest percentage of inappropriate requests (60.7%) was for the treatment of haemorrhagic events, both in medical and surgical settings, without information on baseline haemostatic parameters (PT and PTT) or in the presence of normal values for these parameters. It, therefore, appears that there are situations in which the therapeutic choice seems to be dictated predominantly by the clinical evaluation, independently of laboratory information, since the data were missing completely in 48.3% of the cases. This last figure probably underestimates the real percentage of requests made in the absence of laboratory data, because it does not take into account the 12.3% of inappropriate requests for the prophylaxis of haemorrhagic events in which the absence of PT and/or PTT was combined with normal results for these parameters.
Thus, the lack of laboratory data we found seems to be greater than the 34.1% reported by other Authors\(^a\); this difference is probably due to the different design of the study mentioned, which also evaluated the laboratory data of patients transfused with FFP, with the aid of a specific database. Both results were, however, encouraging in the light of the data from the Sanguis study in 1994, which found that PT data were missing in 84% of the transfusion recipients of FFP in 49 European hospitals\(^b\).

In second place as a cause of inappropriate use of FFP was prophylaxis of bleeding (23.7% of inappropriate requests). Transfer therapy with FFP to correct coagulation test abnormalities before carrying out invasive procedures is a common clinical practice, even if there are no “evidence-based” guidelines on this issue. Haemostasis is a complex process of interactions between pro-coagulant molecules, platelets, natural anticoagulants, the fibrinolytic system and the endothelium. The screening tests of haemostasis prior to surgery are usually PT/INR and PTT; these laboratory tests have been developed to identify causes of bleeding in patients with a demonstrated haemorrhagic diathesis (high pre-test probability) and not to evaluate haemostasis in patients with a negative history for haemorrhage, nor has it been demonstrated that they are able to do so. The transfusion of FFP before an invasive procedure, in order to correct mild to moderate changes in coagulation tests, is not, therefore, able to correct the anomaly or to reduce the perceived haemorrhagic risk\(^c,d,e\).

The use of FFP to correct hypoproteinaemia or for nutritional purposes is greater in developing countries, but it is difficult to find a justification for this practice in the Italian healthcare system\(^f\).

The present study also reveals that inappropriate use of FFP is more common in University hospitals than in smaller, peripheral hospitals, as already reported in the literature\(^g\); this could be due to the greater complexity of cases treated in the University hospitals and the greater amount of blood components distributed by these hospitals, both factors which could limit the possibility of carrying out audits and adhering to guidelines.

Possible limitations of this study are: a) that the audit did not use information technology, which, with a specific database, would have enabled a whole set of diagnostic, clinical and laboratory data to be processed and compared at national and international levels\(^h,i,j,k\); b) the definition of inappropriateness did not take into account the dose of FFP used and, therefore, the possible administration of subtherapeutic doses\(^l\); c) the comparison between the years was not homogeneous, because the data for 2005 were not divided by semester, but referred to the whole calendar year.

The capacity of transfusion structures to make data available systematically and continuously on the use of blood components is an essential feature of clinical governance\(^m\); systematic clinical auditing, particularly if performed at a large scale such as regionally, thus enabling the acquisition of a substantial quantity of data, increases the degree of appropriate behaviours in the field of transfusion therapy, contributes to self-sufficiency, and can direct clinical research towards those sectors in which inappropriate treatment is greatest. Transfusion medicine is, in fact, a transversal medical discipline in which there are few adequately structured trials that provide evidence on the indications and efficacy of transfusion therapy. Appropriately designed clinical trials could modify some decisions, so that they are made on “evidence-based” criteria\(^n\). This is what is happening in the USA, where a multicentre clinical trial should provide evidence on which to decide on the clinical use of FFP in patients with liver disease and INR values of 1.3 - 2, who are candidates for invasive procedures\(^o\); this multicentre study was triggered by a retrospective audit\(^p\), followed by a prospective audit\(^q\), conducted in the same hospital, and a systematic review of the literature on the subject\(^q\).

References


Correspondence: Dr. Giancarlo Maria Liumbruno, Viale Italia, 19 - 57126 Livorno, Italy
e-mail: giancarlo@liumbruno.it

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Plasma derivatives and strategies for reaching self-sufficiency in Liguria: the role of the Transfusion Medicine Service of the Gaslini Institute

Laura Bocciardo1, Marina Martinengo1, Diego Ardenghi1, Tullia Emanuelli2, Enrica Oliva3, Gino Tripodi1

1Servizio di Immunoematologia e Medicina Trasfusionale, Istituto Giannina Gaslini,Genova
2Servizio di Farmacia, Istituto Giannina Gaslini, Genova
3CRCC Liguria, Genova, Italy

Background. Since 2002, Liguria has been part of the Interregional Agreement on Plasma Derivatives (AIP) stipulated among some Regions of north Italy with the aim of contributing to self-sufficiency of the interregional system through exchanges between the facilities lacking products and those with an excess.

In Liguria, the management of plasma derivatives is entrusted to the Regional Centre for Co-ordination and Compensation (CRCC) which, with strategies of compensation, tries to guarantee that the needs for plasma derivatives are covered in the hospitals in its territory.

The Services of Immunohaematology and Transfusion Medicine (SIMT) have a goal of increasing the production of plasma in order to participate actively in achieving regional self-sufficiency.

Methods. The SIMT of the G. Gaslini Institute introduced some strategies aimed at reaching this goal. The increase in the number of donations made with a cell separator, the introduction of multicomponent donations of plasma and platelets and the collection of high concentration platelet concentrates led to a considerable increase category A plasma sent for fractioning. Finally, the implementation of shared guidelines on the use of blood components enabled the clinical use of the plasma collected to be kept under control.

Results and conclusions. The analysis of the trends of consumption of the most widely used plasma derivatives showed an increase in the overall demands, which can be attributed to the paediatric focus of our hospital and to its highly specialised wards.

On the basis of the industrial technical yield, it was possible to calculate the theoretical coverage of the requirements for plasma: this highlighted a better theoretical coverage for albumin but a shortfall of intravenous immunoglobulins. The amount of plasma necessary to meet the theoretical needs was calculated for each plasma derivative, revealing that the derivative requiring the greatest volume of plasma is intravenous immunoglobulins. This finding confirms the change in the "driving product": it is now the consumption of intravenous immunoglobulins that determines the amount of plasma that is sent for industrial processing.

Key words: plasma production, blood derivatives.
Regions on the basis of the amount of plasma supplied by each one of them.

The primary purpose of the Agreement is to combine the efforts of the individual members, in a unanimous and synergistic way, in order to contribute to reaching the priority goal, established by legislation with law n. 219/05 and its subsequent modifications and integrations, that is, national self-sufficiency. Once internal self-sufficiency has been guaranteed, each participating region is committed to contributing to the self-sufficiency of the interregional system, through exchanges between the facilities lacking products and those with excesses.

Being part of the AIP carries economic and organizational advantages both in relations with the pharmaceutical industry and in interregional relations among the Regions adhering to the Agreement. The AIP:
- guarantees greater negotiating power and, therefore, better conditions, with respect to single Regions, with the pharmaceutical industry, that produces drugs derived from the industrial processing of plasma;
- encourages exchanges of blood derivatives between Regions with excesses and those with shortfalls of products at costs lower than market prices;
- guarantees, through the contribution of plasma conferred by each Region, the constant availability of blood derivatives also for Regions that are not able to confer sufficient volumes of plasma for autonomous formation of batches for industrial transformation, thereby limiting these Regions' recourse to the commercial market;
- imposes the standardisation and use of the same production processes (selection of donors, stages of processing and storage, tracking procedures), contributing to the definition of the Plasma Master File (PMF), which is obligatory for safer and more reliable production of blood derivatives;
- may foster a more extensive sharing adoption of guidelines on the use of blood derivatives and close monitoring of the requests from wards, to limit the clinical use of plasma.

**Methods**

**Strategies for increasing the production of plasma to send for industrial fractionation**

Until 2002, plasma in our SIMT was obtained exclusively from two sources: productive plasmapheresis (for category A plasma) and whole blood (for category B plasma and a minimal proportion of category C plasma). Three possible areas of intervention were identified:

- an increase in the plasmapheresis procedures;
- changing the collection of platelets into multicomponent donations of plasma and platelets and, subsequently, the introduction of procedures to collect high-concentration platelet concentrates to increase the volumes of plasma yielded in each procedure;
- the introduction of guidelines on the good use of blood components and close monitoring of the requests from wards, to limit the clinical use of plasma.

**Analysis of the production and internal consumption of plasma**

The data on the production of plasma in the SIMT of the Gaslini Institute in the period 2001-2005 were analysed (volume of each category of plasma), as were the data relating to the proportion of plasma sent for industrial processing and the proportion retained for clinical use.

**Analysis of the consumption of plasma derivatives and of the percentages of theoretical cover of the requirements**

The data relating to the consumption of the plasma derivatives most widely used in the wards in our hospital (albumin, intravenous IgG and antithrombin III) were analysed.
Plasma derivative self-sufficiency in Liguria

On the basis of the yields of each derivative produced per litre of plasma, defined by the pharmaceutical industry in agreement with the AIP (Table I), we calculated the amounts of plasma derivatives obtained from the volume of plasma sent for industrial processing. We, therefore, obtained the percentage theoretical coverage of requirements for each blood derivative guaranteed by the plasma production of the SIMT and identified the derivative whose requirements define the amount of plasma that must be sent to the industry to guarantee internal self-sufficiency.

Table I - Industrial technical yields of plasma derivatives

<table>
<thead>
<tr>
<th>Albumin</th>
<th>I.V. IgG</th>
<th>Antithrombin III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma A</td>
<td>25 g</td>
<td>2.9 g</td>
</tr>
<tr>
<td>Plasma B</td>
<td>25 g</td>
<td>2.9 g</td>
</tr>
<tr>
<td>Plasma C</td>
<td>25 g</td>
<td>2.9 g</td>
</tr>
</tbody>
</table>

Results

Strategies introduced

Starting from the second half of 2002, attempts were made to increase the number of plasmapheresis procedures through a reorganisation of the system in the blood donation room; after 6 months, this organisational model was suspended because of the negative repercussions observed in the management of the blood donations. Other strategies of this type are currently not possible.

However, thanks to new cell separators, since September 2003 it has been possible to replace the collection of platelets by apheresis by multicomponent donations of plasma and platelets, without this causing significant reductions in the yields of platelet collections.

In the two years 2004-2005, the mean volume of plasma from plasmapheresis increased slightly, by modifying the programme of the cell separators. In September 2006, the collection of high-concentration platelet concentrates began, leading to a significant increase in the volume of plasma collected.

In December 2004, the Committee for the Good Use of Blood approved guidelines on the use of blood components. Thanks to these guidelines, a more rigorous control of the requests for plasma for clinical use was possible. In contrast, internal guidelines, approved by Committee for the Good Use of Blood, on the use of albumin, intravenous immunoglobulins and antithrombin III are still not available, although they have been the subject of discussion and debate for some time now.

Plasma production

The analysis of the data for the period 2001-2005 showed a considerable increase in the volume of plasma produced (Figure 1); the production increased from 1,560 litres in 2001 to 1,720 and 1,730 litres in 2002 and 2003, respectively; there were further increases in production in 2004 and 2005, reaching 1,830 and 1,890 litres of plasma, respectively. From the destination of the plasma that, after the validation process, enters the distribution circuit, it can be seen that during the 5 years considered, the volume of category B and C plasma did not vary significantly, ranging between 1,008 and 1,096 litres a year. In contrast, the increase in plasma from apheresis (plasma A) sent for industrial fractionation was constant and appreciable, passing from 267 litres in 2001 to 508 litres in 2005.

The observed increase was not, overall, related to the increase in the number of apheresis procedures which, particularly in the three years 2003-2005, was basically stable and lower than the values in the preceding two years (Table II), but rather, could be attributed to the optimisation of the collection procedures carried out using cell separators. Finally, it can be seen that during the period studied, the volume of plasma used for clinical purposes remained relatively steady (Table III and Figure 2).

This evaluation of the production of plasma in the Region of Liguria enabled a quantification of the role played by the SIMT of the Gaslini Institute within the Region; during 2005, our Institute collected 8% of the total regional collection (Figure 3) of plasma from ordinary donations and 16% (Figure 4) of the plasma collected by cell separators.
Table II - Productive apheresis procedures carried out in the SIMT

<table>
<thead>
<tr>
<th>Year</th>
<th>N. of plasmapheresis procedures</th>
<th>N. of plateletpheresis procedures</th>
<th>N. of plasma-plateletpheresis procedures</th>
<th>Total n. of productive apheresis procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>882</td>
<td>922</td>
<td>0</td>
<td>1,804</td>
</tr>
<tr>
<td>2002</td>
<td>1,041</td>
<td>874</td>
<td>0</td>
<td>1,915</td>
</tr>
<tr>
<td>2003</td>
<td>727</td>
<td>288</td>
<td>671</td>
<td>1,666</td>
</tr>
<tr>
<td>2004</td>
<td>808</td>
<td>0</td>
<td>975</td>
<td>1,783</td>
</tr>
<tr>
<td>2005</td>
<td>866</td>
<td>3</td>
<td>887</td>
<td>1,756</td>
</tr>
</tbody>
</table>

Table III - Distribution of plasma (in litres) in the period 2001-2005

<table>
<thead>
<tr>
<th>Year</th>
<th>Total plasma distributed</th>
<th>Plasma for clinical use</th>
<th>Class A plasma sent for industrial fractionation</th>
<th>Class B+C plasma sent for industrial fractionation</th>
<th>Total plasma sent for industrial fractionation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>1,520</td>
<td>245</td>
<td>287</td>
<td>1,008</td>
<td>1,275</td>
</tr>
<tr>
<td>2002</td>
<td>1,619</td>
<td>286</td>
<td>308</td>
<td>1,025</td>
<td>1,333</td>
</tr>
<tr>
<td>2003</td>
<td>1,621</td>
<td>250</td>
<td>560</td>
<td>1,011</td>
<td>1,371</td>
</tr>
<tr>
<td>2004</td>
<td>1,774</td>
<td>290</td>
<td>461</td>
<td>1,023</td>
<td>1,484</td>
</tr>
<tr>
<td>2005</td>
<td>1,864</td>
<td>260</td>
<td>508</td>
<td>1,096</td>
<td>1,604</td>
</tr>
</tbody>
</table>

The overall normalised needs, obtained by summing the specific needs, are also represented graphically. As can be seen from this latter graph, the overall requirements are increasing constantly (with a mean annual increase of about 6%), even if the specific requirements for each plasma derivative are obviously dependent on the population of patients in the corresponding year. In particular, it can be seen that in 2002, the maximum relative need for intravenous IgG (8,445 g) corresponded to the minimum requirements for both albumin (34,190 g) and antithrombin III (386,000 U.I.); similarly, in 2004, a minimum relative need for intravenous IgG (6,476 g) corresponded to maximum requirements for both albumin (44,000 g) and antithrombin III (550,000 U.I.).

Covering the needs

Starting from the industrial technical yields (Table I), it is clear that the increased production of plasma in our SIMT improved the theoretical coverage of the need for albumin, which increased from 82.6 % in 2001 to 91.6 % in 2005. The data on intravenous IgG were less comforting: the theoretical coverage of needs, which in 2001 was 61.4 %, decreased to 54.2 % in 2005 (Table V).

As far as concerns antithrombin III, we calculated that 23.9 % of the requirements were covered in 2005. It should be noted that this is a partial result in that...
Plasma derivative self-sufficiency in Liguria

Figure 3 - Pie chart of the production of plasma from ordinary blood donations in the Region of Liguria in 2005

Figure 4 - Pie chart of the production of class A plasma from apheresis in the Region of Liguria in 2005
Table IV - Consumption of plasma derivatives by the Gaslini Institute

<table>
<thead>
<tr>
<th>Year</th>
<th>Consumption of albumin (g)</th>
<th>Consumption of intravenous IgG (g)</th>
<th>Consumption of antithrombin III (UI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>36,580</td>
<td>6,024</td>
<td>405,000</td>
</tr>
<tr>
<td>2002</td>
<td>34,190</td>
<td>8,445</td>
<td>396,000</td>
</tr>
<tr>
<td>2003</td>
<td>43,290</td>
<td>7,830</td>
<td>388,000</td>
</tr>
<tr>
<td>2004</td>
<td>44,000</td>
<td>6,476</td>
<td>550,000</td>
</tr>
<tr>
<td>2005</td>
<td>43,762</td>
<td>8,579</td>
<td>530,500</td>
</tr>
</tbody>
</table>

Table V - Theoretical coverage of the requirements of plasma derivatives

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>82.62%</td>
<td>97.47%</td>
<td>79.18%</td>
<td>84.32%</td>
<td>91.63%</td>
</tr>
<tr>
<td>Intravenous IgG</td>
<td>61.38%</td>
<td>45.78%</td>
<td>50.78%</td>
<td>66.45%</td>
<td>54.22%</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>23.94%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5 - Requirements for albumin, intravenous IgG and antithrombin III, normalised to 2001 levels. The virtual overall requirements, calculated as the sum of the three products, is also reported, again normalised to 2001 levels.

antithrombin III was distributed after third party processing starting from March of the same year.

Finally, for each blood derivative considered, we evaluated the amount of plasma necessary to meet the theoretical requirements.

The graph in figure 6 shows the trends in the amounts needed for each of these derivatives each year.

It is clear from this that the blood derivative giving rise to the greatest request for plasma is intravenous IgG.

Discussion
In line with the national plan for reaching self-sufficiency in plasma derivatives, the SIMT of the Gaslini Institute for children defined a plan to increase the volume of plasma to send for industrial fractionation as an objective of its Quality System. This led to the definition of new procedures for both production and clinical use of plasma.

The increase in plasma production was obtained without compromising the primary objective of the SIMT.
The number of litres of plasma necessary to meet the demands for albumin, intravenous IgG and antithrombin III over the years studied.

In September 2006, the technique of collecting high-concentration platelet concentrates during multicomponent donations was introduced. It is estimated that this technique will further increase the production of category A plasma by about 400 litres/year. In addition, considering that about 25% of the units of plasma are assigned to low weight patients, the use of paediatric units of 50 mL (units for clinical use are currently fractioned into subunits of 100-150 mL) will not only further reduce the risk of transfusions (by enabling plasma from the same donor to be used for more than one transfusion) but also save an estimated volume of more than 40 litres of plasma each year.

The implementation of shared guidelines on the use of the blood components has, finally, allowed the clinical use of the plasma collected to be kept under control.

Although the production of plasma has increased considerably in the last few years, it is not yet sufficient to meet the Institute’s requirements for blood derivatives which are, in fact, guaranteed by the policy of the CRCC.

The consumption of blood derivatives in our Institute is high because the patients are children and because it contains numerous highly specialised wards. The high consumption of plasma derivatives is only partly under control, because of the lack of agreed guidelines within the Institute. However, the introduction of regional guidelines on the use of albumin in 2002 may have contributed to the fact that there was a relatively limited increase in the consumption of albumin, in the three years 2003-2005. On the other hand, the use of intravenous IgG has always been such that the theoretical coverage of needs is poor. A recent review of the literature showed that the number of publications on the use of intravenous IgG continues to increase rapidly, although in reality there is little evidence obtained from randomised, controlled clinical trials. This has led to a notable increase in off-label prescriptions, estimated, by the Food and Drug Administration, to account for between 50% and 70% of all prescriptions, even in organisational settings such as in America, in which the use of guidelines is consolidated. Although the increase in the consumption of intravenous IgG is also due to the expanded indications for use in certain number of clinical conditions, it is clear that the Institute must implement an agreed document regulating the use of this product. The increased consumption of immunoglobulins is a phenomenon present nationwide; our data do not, therefore, reflect the fact that our Institute cares for paediatric patients, but rather confirm the change in the ‘driving product’, already noted by other researchers. The consumption of albumin is now no longer significantly greater than that of the other plasma derivatives, such as...
that it is mainly the consumption of intravenous IgG that determines the amount of plasma to be sent for industrial processing.

Precisely because of its widespread clinical use, the goal of meeting demands for intravenous IgG is difficult to achieve, since it would require an annual increase of about 1,350 litres in the production of plasma, which is equivalent to about 84% of the current production. If such an aim were to be reached, it would be of great financial importance, given that the cost of intravenous IgG is much higher than that of albumin. It should be remembered that the greater productive efficiency, achieved in 2005-2006, by the company that processes the plasma, with the current yield of intravenous IgG being 3.1 g/L, could contribute to greater availability of the product, without lowering the level of purity of the drug.

As far as concerns antithrombin III, the problem of the low theoretical coverage of requirements (23.94%) derives from the fact that this drug is produced only from class A plasma. At a regional level, this makes Liguria dependent on the production in other regions belonging to the AIP, which collect a larger number of donations with cell separators.

In order to contribute to self-sufficiency in plasma derivatives, it is essential to implement regionally-based strategies and, in some circumstances, interregional ones. These strategies must take into account factors related to the type of population of the donors, logistical aspects and agreements between the Associations that promote the donation of blood. For this reason the role of the intra and extra-regional co-ordination, defined by law (Legislative Decree. 219/05), is fundamental.

Conclusions

The analysis of the data collected leads to the conclusion that, although the production of plasma in the last four years has increased and the consumption for clinical use has remained roughly stable, the volume of plasma sent for industrial processing is insufficient to meet the requirements for plasma derivatives: this is particularly obvious for intravenous IgG, which are heavily requested.

The quantification of this lack of coverage enabled us to plan procedures to reduce the shortfalls: the strategies decided were to further increase the production of plasma and in particular the volume of plasma collected using a cell separator, and to optimise the clinical use of plasma and plasma derivatives.

Our analysis also led us to conclude that the optimisation of plasma production will never be sufficient to cover the notable increase in the consumption of intravenous IgG in our Institute: it is, therefore, extremely important to evaluate whether the increased requests are due to an increase or improvement in health care services delivered, or whether they are due to inappropriate consumption of these plasma derivatives.

In the light of the most authoritative international recommendations, collaboration with clinicians is essential in order to optimise the use of plasma products and must have the concrete outcome of the production of guidelines and the control of the appropriateness of the requests.

In fact, the good use of plasma derivatives, achieved by following appropriate guidelines, has obvious implications for the safety of transfusions, but also has clear financial consequences.

The SIMT of the Gaslini Institute, thanks to its rationalisation of plasma production and optimisation of the consumption of plasma derivatives and plasma for clinical use, is participating actively in the attempt to reach regional self-sufficiency. Nevertheless, the CRCC is essential in order to evaluate the various realities in the regional context.

References

Removal kinetics of exchange transfusion

Roberto Reverberi, Lorenzo Reverberi

Servizio di Immunematologia e Trasfusionale, Arcispedale S. Anna, Azienda Ospedaliera - Universitaria di Ferrara, Italy

Exchange transfusion has just a few elective indications: the therapy of haemolytic disease of the newborn (HDN) and cases in which red cell exchange is appropriate but the child is too small to use a cell separator.

Exchange transfusion has several positive effects in the therapy of HDN: it removes the neonatal red cells, which are destined to be destroyed and hence to generate bilirubin, the already produced bilirubin, and the offending antibody. Moreover, exchange transfusion also corrects anaemia, if present.

Physiological bases of exchange transfusion in HDN

The offending antibody is an IgG and has a substantial extravascular distribution (about 60%). As the equilibration between the intravascular and extravascular compartments is slow (≈ 1-3%/hour), removal of the antibody through the exchange may only be partial and the residual or newly produced neonatal red cells are still exposed to the risk of haemolysis. It is, therefore, justified to remove as many neonatal red cells as possible and to maintain an elevated total haemoglobin concentration with compatible adult red cells, so as to inhibit the autologous production from the bone marrow. The bilirubin in the extravascular space is only a small part of the total and, although equilibration is fast, exchange transfusion is not particularly successful: rebound is immediate, so that at the end of an exchange during which 87% of neonatal red cells are removed, the concentration of bilirubin is still 60% of that at the start of the exchange. It is, therefore, advisable to prevent the production of bilirubin by removing the “doomed” redcells intime.

The removal of red cells

Alexander S. Wiener (1907-1976), co-worker of Karl Landsteiner in the discovery of the Rh factor (1940) and pioneer of the therapy of HDN, asserted that he and Wexler had been the first to derive the formula which describes the removal of red cells during exchange transfusion:

\[ \text{Residual fraction} = e^{\frac{-v}{V}}. \]

where \( v \) is the total exchanged volume, \( V \) is the patient’s blood volume and \( e \) is the transcendental number, base of the natural logarithm. However, he later stated that his formula was not correct because it did not take into consideration the difference between the patient’s haematocrit and that of the transfused blood. Moreover, according to others, even when the two haematocrits happen to be the same, the formula should be modified to account for the difference between venous haematocrit and body haematocrit. Wiener also stated that it was obvious that, for an equal exchanged volume, exchange transfusion would be more efficient, the more the neonate was anaemic. Taken together, these opinions risk misleading the reader seriously.

Wiener’s formula describes a continuous flow exchange. However, during exchange transfusion, neonatal blood is replaced by donor blood, withdrawing and transfusing alternatively 5-20 mL of blood. The exchange is, therefore, intermittent. It can be demonstrated (see Appendix A) that when blood is first withdrawn and then transfused, at each cycle:

\[ \text{Residual fraction} = \left(1 - \frac{s}{kV}\right)^n, \]  

where \( s \) is the volume exchanged at each cycle (cycle volume), \( V \) is the patient’s blood volume, \( k \) is the ratio between body and venous haematocrit (≈ 0.91) and \( n \) is the number of exchange cycles.
Analogously, when blood is first transfused and then withdrawn:

\[ Residual\ fraction = \left(1 - \frac{x}{k(V + x)} \right)^y, \quad (2) \]

In Appendix B, the reader will find instructions for programming a spreadsheet to calculate the residual fraction according to the cycle volume and the neonate's blood volume. Some exemplary data are shown in Table I.

As can be easily noted, the efficiency of the exchange is greater when blood is first withdrawn. The difference is proportional to the volume exchanged at each cycle. The cycle volume influences the efficiency of the exchange in a peculiar way: when blood is first withdrawn, efficiency is proportional to the cycle volume, whereas when blood is first transfused, efficiency is inversely proportional to the cycle volume. However, as the cycle volume rarely exceeds 10% of the total blood volume, the differences are limited to a few percentage points.

Formulae (1) and (2) do not include references to the patient's hematocrit, nor to the hematocrit of the transfused blood: contrary to the above cited opinions, the residual fraction only depends on the terms represented in the two formulae.

Absolute amount of neonatal red cells

However, we should be more interested in the absolute amount of the residual neonatal red cells, rather than the fraction. In this case it is clear that, if the neonate is anemic, during the exchange it is possible to fall under a minimum target level of neonatal red cells earlier.

Incidentally, this was the probable meaning of Wiener's sentence cited above.

Figure 1 compares the cases of two hypothetical neonates, with the same weight (blood volume) but initial hematocrits of 25% and 50%. If our aim is to leave no more than 9 mL of neonatal red cells (about 10% of the normal initial volume), the target is met in the first case after 14 cycles of exchange and in the second after 20 cycles.

The neonate's venous hematocrit at the end of the procedure

During the exchange transfusion, neonatal red cells are progressively substituted by the transfused cells. The venous hematocrit after \( n \) cycles of exchange (\( H_n \)) principally depends on the hematocrit of the transfused blood (\( H_D \)) and, moreover, the volume exchanged at each cycle (\( s \)), the number of cycles (\( n \)), and the neonate's initial venous hematocrit (\( H_V \)), according to the following formula (see Appendix A):

\[ H_n = \left(1 - \frac{x}{kV} \right) \times H_V + \left(1 - \left(1 - \frac{x}{kV} \right) \right) \times H_D \quad (3) \]

The above formula is appropriate when the exchange begins withdrawal of blood. The analogous formula for the opposite case is given in Appendix A.

Figure 2 shows the kinetics of the substitution of the transfused blood for the neonatal red cells and the changes of the patient's venous hematocrit during the exchange transfusion.

Table I - Fraction of neonatal red cells remaining after 1, 2, 5, 10, and 20 cycles during the exchange transfusion. It is assumed that the volume exchanged at each cycle is 10% of the total blood volume. Columns "Withdrawal first" and "Transfusion first" show the results when the exchange begins with withdrawal or transfusion, respectively. For the sake of comparison, the column on the right shows the results of an isovolumetric continuous flow exchange. Its efficiency is intermediate between the other two.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Total exchanged volume (% of the blood volume)</th>
<th>Residual fraction (%) of the neonatal red cells</th>
<th>Intermittent flow</th>
<th>Continuous flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Withdrawal first</td>
<td>Transfusion first</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>89.0</td>
<td>90.0</td>
<td>89.5</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>79.2</td>
<td>81.0</td>
<td>80.1</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>55.9</td>
<td>59.1</td>
<td>57.4</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>31.2</td>
<td>34.9</td>
<td>32.9</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
<td>8.8</td>
<td>12.2</td>
<td>10.8</td>
</tr>
</tbody>
</table>
Removal kinetics of exchange transfusion

Figure 1 - Effect of the neonate’s initial venous haematocrit on the residual quantity of neonatal red cells during the exchange transfusion. It is assumed that the blood volume of the neonate is 200 mL, the volume of the exchange at each cycle is 20 mL (10%), and blood is first withdrawn and then transfused. The solid curve with closed symbols refers to an initial venous haematocrit of 25%. The dotted curve with open symbols refers to a haematocrit of 50%.

Figure 2 - Kinetics of the substitution of donor red cells for the neonatal red cells and of the venous haematocrit during the exchange transfusion. The conditions of the procedure are the same as those imagined in figure 1 (initial venous haematocrit of the neonate: 25%). The haematocrit of the transfused blood is 50%. The solid curve with closed symbols indicates the residual volume of neonatal red cells. The dotted curve with solid symbols indicates the transfused red cells circulating in the patient. The solid curve with open symbols indicates the patient’s venous haematocrit.
Choosing the haematocrit of the blood to be transfused

It has been noted that the haematocrit of the transfused blood does not influence the efficiency of the procedure and, therefore, it may be chosen on the basis of other considerations: the haematocrit should not be too low, in order not to induce a state of anaemia and to correct it, if present; an elevated haematocrit at the end of the procedure exerts an inhibitory effect on autologous marrow production, which is in any case destined to precocious destruction. On the other hand, an excessive haematocrit of the transfused blood limits the total exchanged volume, lest the neonate be made too polycythaemic. This entails the risk of an insufficient exchange. Since the 1950s, it has been customary to concentrate the reconstituted whole blood up to a haematocrit of about 50%. This practice was confirmed by the recent Italian guidelines. Probably, this choice stems from the fact that the normal neonatal haematocrit is about 50%. Such an elevated haematocrit is due to the greater affinity for oxygen by the foetal haemoglobin that, therefore, has a lower oxygen carrying capacity to the tissues. In fact, haematocrit rapidly decreases in the weeks following birth. After about 2 months, even though the substitution by adult haemoglobin is not complete, the haematocrit has fallen to about 35%. Therefore, a haematocrit of 50% is not necessary and it could be decreased to 45% or less, so as to profit from the greater volume of reconstituted whole blood available to increase the removal of neonatal red cells or bilirubin.

Exchange transfusion in the therapy of sickle cell anaemia

Exchange transfusion is used preferentially or as an alternative to simple transfusion in a number of indications in children with sickle cell anaemia. In adults or bigger children, automated red cell exchange is the preferred procedure (this topic will be dealt with in the next article of this series), but the manual exchange of whole blood may be necessary for smaller children, given the limitations in the extracorporeal volume. In this case, the removal kinetics is the same as that described above. The difference lies in the target, which is not to remove as many autologous red cells as possible, but to reduce the percentage of haemoglobin S without increasing the haematocrit excessively, in order not to cause a high blood viscosity.

The relevant parameters are, therefore, the percentage of haemoglobin S and the venous haematocrit. This latter can be calculated from (3). The first part of equation (3) represents the contribution \( H_r \) of the residual autologous red cells (see Appendix A):

\[
H_r = \left(1 - \frac{x}{k} \right) \times H_v
\]

Therefore, the percentage of haemoglobin S after \( n \) cycles \( H_bS_n \) will be:

\[
H_bS_n = \frac{H_r}{H_v} \times H_bS_0
\]

where \( H_bS_0 \) is the initial percentage of haemoglobin S.

Limitations of the formulae

Most of the terms contained in the formulae can be measured with precision. However, blood volume is, usually, only estimated with the aid of a formula and the value of the correction for the body haematocrit is taken from the literature. In this regard, however, it should be noted that the value may differ from the normal significantly, in case of splenomegaly.

Moreover, the formulae assume that:
- there is no dead space in the transfusion set used for the exchange;
- the transfused blood mixes immediately in the circulation;
- the blood volume does not change during the procedure;
- all transfused red cells survive in the circulation (at least till the end of the exchange);
- the patient’s red cells are not destroyed during the procedure.

As regards the first assumption, after the transfusion step, before blood is again withdrawn, it is advisable to aspirate a small volume of blood from the patient and to reinfuse it. The second and third assumptions are plausible. The fourth is also plausible, in the case of HDN, because the neonate’s reticuloendothelial system should preferentially remove the sensitised autologous red cells. Finally, the fifth assumption depends on the duration of the procedure.

Conclusions

The exchange is more efficient if blood is first withdrawn and if the volume exchanged at each cycle is large. However, when the cycle volume does not exceed 10% of the patient's
Appendix A – Derivation of the formulae

The fraction of neonatal red cells removed in the exchange transfusion

If we denote the volume exchanged at each cycle with \( s \) and the blood volume of the patient with \( V \), then the fraction removed at each cycle, when blood is first withdrawn, should be \( \frac{V}{s} \).

However, the red cells are more concentrated in the venous blood than in the whole vascular system, so that:

\[
HB = \frac{V}{k} \times H_v
\]

where \( HB \) is the body haematocrit, \( H_v \) the venous haematocrit and \( k \) is the correction factor (= 0.91)7.

Let \( RB \) be the total volume of the patient’s red cells:

\[
RB = \frac{V}{k} \times H_v \times V
\]

Let \( RS \) be the volume of the patient’s red cells in the volume exchanged at each cycle:

\[
RS = \frac{sk}{100} \times H_v \times V
\]

Then the removed fraction will be equal to

\[
\text{Removed fraction} = \frac{kV}{s}
\]

and the residual fraction will be:

\[
\text{Residual fraction} = 1 - \frac{kV}{s}
\]

At the next cycle, the volumes exchanged will be the same, but the removal will concern the residual amount only \( \left( 1 - \frac{kV}{s} \right) \), therefore the residual fraction after the second cycle will be:

\[
\left( 1 - \frac{kV}{s} \right) \times \left( 1 - \frac{kV}{s} \right), \text{ or } \left( 1 - \frac{kV}{s} \right)^2.
\]
It is easy to see that after \( n \) cycles:

\[
\text{Residual fraction} = \left(1 - \frac{s}{kV}\right)^n \quad (2)
\]

When blood is first transfused, on the basis of very similar considerations to those above, it can be derived that:

\[
\text{Residual fraction} = \left(1 - \frac{s}{k(V + s)}\right)^n \quad (3)
\]

In the above formulae, only the correction for the difference between venous and body haematocrit was used. However, a further correction is advisable when the haematocrit is measured by centrifugation because of the small amount of plasma (\( \approx 2\% \)) that remains entrapped in the red cell column\(^8\). In this case \( k = 0.91 \times 0.98 = 0.89 \).

**Absolute amount of neonatal red cells remaining after the exchange transfusion**

The volume of the neonatal red cells remaining after the exchange cycles \( R_n \) can be derived from formula (2):

\[
R_n = R_b \times \left(1 - \frac{s}{kV}\right)^n
\]

or formula (3), as appropriate:

\[
R_n = R_b \times \left(1 - \frac{s}{k(V + s)}\right)^n
\]

\( R_n \) can be derived from (1). The removed volume can be easily calculated by difference.

**Venous haematocrit after the exchange transfusion**

Let us consider the case in which blood is first withdrawn. Let \( a = \frac{s}{kV} \) and \( b = 1 - a \).

At each cycle, a fraction equal to \( a \) of the donor's red cells present in the circulation will be removed, whilst a volume \( D_j \) will be added.

Table II shows the situation in the first cycles of an exchange transfusion.

As can be seen from the far right column, at each cycle, a term to the power \( n - 1 \) is added in parentheses.

By definition, we know that:

\[
H_b = \frac{D_a}{s} \times 100 = \frac{D_b}{(1 - b)kV} \times 100
\]

where \( H_b \) is the haematocrit of the transfused blood, and that:

\[
H_d = \left(1 + b + b^2 + \ldots + b^{n-1}\right) \times \frac{D_s}{kV} \times 100
\]

where \( H_d \) is the venous haematocrit (in the patient’s circulation) of the transfused red cells after cycle \( n \).

Therefore:

\[
H_n = (1 - b) \times \left(1 + b + b^2 + \ldots + b^{n-1}\right) \times H_b = (1 - b^n) \times H_d
\]

Table II - Volumes of donor red cells (VGR) exchanged during the exchange transfusion. At each cycle, blood is first withdrawn and then transfused. In the first cycle, no donor red cells are in the circulation during the withdrawal step. \( D_0 \) is the volume of donor red cells transfused at each cycle.

<table>
<thead>
<tr>
<th>CYCLE</th>
<th>Removed V_{in}</th>
<th>Remaining V_{in}</th>
<th>Final V_{in}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>( a \times D_j )</td>
<td>( b \times D_j )</td>
<td>( D_j + b \times D_j )</td>
</tr>
<tr>
<td>3</td>
<td>( a \times (1 + b + b^2) \times D_j )</td>
<td>( (b + b^2) \times D_j )</td>
<td>( (1 + b + b^2) \times D_j )</td>
</tr>
<tr>
<td>4</td>
<td>( a \times (1 + b + b^2 + b^3) \times D_j )</td>
<td>( (b + b^2 + b^3) \times D_j )</td>
<td>( (1 + b + b^2 + b^3) \times D_j )</td>
</tr>
<tr>
<td>...</td>
<td>( a \times (1 + b + b^2 + \ldots + b^{n-1}) \times D_j )</td>
<td>( (b + b^2 + \ldots + b^{n-1}) \times D_j )</td>
<td>( (1 + b + b^2 + \ldots + b^{n-1}) \times D_j )</td>
</tr>
</tbody>
</table>
Removal kinetics of exchange transfusion

By substitution, the following formula is obtained:

\[ H_x = \left( 1 - \frac{s}{H_x} \right) \times H_v. \]

In the same way, we can derive the venous haematocrit of the patient’s red cells after the exchange transfusion (\(H_p\)):

\[ H_p = \left( 1 - \frac{s}{H_p} \right) \times H_v. \]

Therefore, at the end of the cycles of exchange transfusion, the total venous haematocrit will be the sum of the two partial ones:

\[ H_s = \left( 1 - \frac{s}{H_s} \right) \times H_v + \left( 1 - \frac{s}{H_s} \right) \times H_p. \]

This formula allows the calculation of the venous haematocrit of the patient during the exchange transfusion (\(H_s\)), starting from the patient’s venous haematocrit before the procedure (\(H_v\)) and the haematocrit of the transfused blood (\(H_p\)).

Analogously, when blood is first transfused, the following formula can be derived:

\[ H_s = \left( 1 - \frac{s}{H_s} \right) \times H_v + \left( 1 - \frac{s}{H_s} \right) \times H_p. \]

Appendix B – Programming a spreadsheet

Spreadsheets are programmes used to perform calculations, especially on large data series.

The most renowned is Excel, which is part of the MS Office (Microsoft) package.

However, the following instructions are equally valid for other similar programmes, such as OpenOffice.org Calc and Gnumeric. As these are less well known, here is a brief description.

OpenOffice.org Calc

This is part of the OpenOffice.org suite. It is freely downloadable from http://www.openoffice.org/ and is available for Windows (98 and later), Mac OS X, Linux and other operating systems. Calc can open, modify, and save files in Excel and other formats, besides its own native one. The available functions are similar to those of Excel.

Gnumeric

Gnumeric is part of the GNU project. It can be freely downloaded from http://www.gnome.org/projects/gnumeric/ and is available in versions for Windows (XP, 2000 or later) and Linux. Gnumeric is able to open, modify and save files in Excel and other formats. Available functions are similar to those of Excel but, in addition, Gnumeric offers a series of statistical tests.

The following instructions only presuppose a basic knowledge of how to use a personal computer.

Instructions

Open the spreadsheet. The programme automatically proposes a new sheet (table), with rows progressively numbered starting from 1 and columns alphabetically labelled starting from A.

Each cell is identified by a couple of column and row identifiers: e.g., the top leftmost cell is A1; the fourth from top in the B column is B4 and so on. Each cell may contain numerical values, formulae or explanatory text. This last is useful for reminding the user of the meaning of the adjacent values or formulae.

Calculation of the residual fraction and absolute quantity of the patient’s red cells

Enter the text listed in Table III into the appropriate cells. Enter the values listed in Table IV.

* Italian readers using the localized (Italian) versions of the spreadsheets should follow the instructions in the Italian translation of this paper, which is available on line at http://www.transfusionmedicine.org/. Briefly, “potenza” should be substituted for “power” and “;” should be substituted for “,”.
Table III - Explanatory text ("labels") to be entered into the specified cell

<table>
<thead>
<tr>
<th>Cell</th>
<th>Text to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Kinetics of exchange transfusion</td>
</tr>
<tr>
<td>A3</td>
<td>Patient’s blood volume (mL)</td>
</tr>
<tr>
<td>A4</td>
<td>Volume of each exchange cycle (mL)</td>
</tr>
<tr>
<td>A5</td>
<td>Patient’s initial venous haematocrit (%)</td>
</tr>
<tr>
<td>A6</td>
<td>Haematocrit of the blood to be transfused (%)</td>
</tr>
<tr>
<td>A7</td>
<td>Correction factor for the body haematocrit</td>
</tr>
<tr>
<td>A9</td>
<td>Cycle</td>
</tr>
<tr>
<td>B9</td>
<td>Exchanged volume (% of the blood volume)</td>
</tr>
<tr>
<td>C9</td>
<td>Residual fraction (%)</td>
</tr>
<tr>
<td>D9</td>
<td>Residual quantity (mL)</td>
</tr>
</tbody>
</table>

Table IV - Values to be entered into the specified cells. Please enter the numerical values only, not the text.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Value to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3</td>
<td>Blood volume of the patient (mL), e.g. 200</td>
</tr>
<tr>
<td>B5</td>
<td>Initial haematocrit of the patient, e.g. 25</td>
</tr>
<tr>
<td>B6</td>
<td>Haematocrit of the transfused blood, e.g. 50</td>
</tr>
<tr>
<td>B7</td>
<td>Correction factor for body haematocrit: 0.91</td>
</tr>
</tbody>
</table>

Enter the numbers from 0 to 25 into the cells from A10 to A35. These numbers represent the cycles of the exchange transfusion. The number of cycles can be increased further at will. Enter the formulae listed in Table V.

Table V - Formulae to be entered into the specified cells. Formulae should be entered as plain text, without forgetting the initial "=" sign

<table>
<thead>
<tr>
<th>Cell</th>
<th>Formula to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10</td>
<td>=$B$4/$B$3<em>A10</em>100</td>
</tr>
<tr>
<td>C10</td>
<td>=100*POWER(1-$B$4/$B$3/$B$7,A10)</td>
</tr>
<tr>
<td>D10</td>
<td>=$B$5/100*$B$7*$B$3*C10/100</td>
</tr>
</tbody>
</table>

Right click on cell B10; choose "Copy" from the pop-up menu; paste the cell ("Paste") on cells B11 to B35 (or further on, if numbering continues past A35). The spreadsheet transposes the references to the cells contained in the formulae in an appropriate way. Calculated values appear immediately. Row 10 contains the starting values, before the exchange. Cells B10-B35, C10-C35 and D10-D35 contain the total exchanged volume, expressed as a percentage of the blood volume, the residual fraction (%) and the residual absolute amount (mL) of the patient’s red cells, respectively. If the user changes one or more values in cells B3-B7, the results in the cells B10-B35, C10-C35 and D10-D35 are immediately recalculated by the programme. When blood is first transfused, the formula to be entered into cell B10 is:

=100*POWER(1-$B$4/$B$3/$B$7,A10).

The other instructions are the same.

Table VI - Instructions to calculate the patient’s venous haematocrit after the exchange transfusion.
(The following instructions presuppose that the previous ones have been correctly performed).

<table>
<thead>
<tr>
<th>Cell</th>
<th>Text to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>E9</td>
<td>Patient’s venous haematocrit</td>
</tr>
</tbody>
</table>

Copy cell E10 into cells E11-E35 (or further on, if numbering continues past A35). Cells E10-E35 will show the venous haematocrit of the patient after each exchange cycle. If blood is first transfused, the formula to be entered into E10 is:

=SB$5*POWER(1-$SB$4/($SB$3+$SB$4)/$SB$7,A10)+$SB$6*(1-POWER(1-$SB$4/($SB$3+$SB$4)/$SB$7,A10)).

Calculation of the percentage of haemoglobin S
(The following instructions presuppose that the previous ones have been correctly performed).
Enter the text, the value, and the formula contained in Table VII.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Text to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>Initial percentage of HbS</td>
</tr>
<tr>
<td>F9</td>
<td>Residual percentage of HbS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Value to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>B4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula to be entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>F10 =</td>
</tr>
</tbody>
</table>

Copy cell F10 into cells F11-F35 (or further on, if numbering continues past A35). Cells F10-F35 will show the percentage of haemoglobin S after each exchange cycle. If blood is first transfused, the formula to be entered into F10 is:

=SB5^POWER(1-SB5+SB5/|B5|+SB5,SB5,SB5,SB5,SB5/|B5|*SB5).
An acute haemolytic transfusion reaction due to anti-Jkα

Maria Antonietta Villa1, Marilyn Moulds2, Elena Beatrice Coluccio1, Mara Nicoletta Pizzi1, Cinzia Paccapelo1, Nicoletta Revelli1, Fernanda Morelati1, Francesca Truglio1, Maria Cristina Manera1, Alberto Tedeschi1, Maurizio Marconi1

1 U.O. Centro Trasfusionale e di Immunoematologia, Dip. di Medicina Rigenerativa, Fond. Osp. Maggiorone Policlinico, Mangiagalli e Regina Elena, Ist. di Ricovero e Cura a Carattere Scientifico, Milan, Italy
2 Education Services, Immucor Inc., Norcross, GA, USA
3 U.O. Medicina Interna II, Fond. Osp. Maggiorone Policlinico, Mangiagalli e Regina Elena, Ist. di Ricovero e Cura a Carattere Scientifico, Milan, Italy

CASE REPORT

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Introduction

The Kidd system antibodies are characteristically difficult to detect. They show variability in immunoglobulin class, subclass and serological characteristics. They are generally detected by an antiglobulin test, using a polyspecific antiglobulin or complement antiserum. Often, the antibodies are only detected using cells with a double dose (homozygous) expression of Kidd antigens, enzyme-treated cells or by using sensitive immunohaematological techniques.

Case report

A 73-year old woman, with a history of two pregnancies and no red cell transfusions, was admitted to our hospital. She had severe anaemia, cirrhosis related to hepatitis C virus infection, cryoglobulins, mild ascites infection and mild renal failure. There were no reported incidents of red cell immunisation.

On admission, her haemoglobin concentration was 7.5 g/dL and her haematocrit was 23%.

On day 3 of hospitalisation, the first red blood cell (RBC) transfusion was required and performed (day 0). The patient’s group was A1B, Rh+.

The antibody screening was negative, using our standard automated method for pre-transfusion testing (AutoVue System with Ortho BioVue microcolumn, Ortho-Clinical Diagnostics, Inc., Raritan, New Jersey, USA) with polyspecific anti-human globulin (anti-IgG+C3d) and an EDTA plasma sample. She received an ABO/Rh compatible standard packed red cell unit (about 170 mL of packed red cells) with the Type & Screen procedure, as indicated by national legislation.

The post-transfusion level of haemoglobin was 8.4 g/dL.

Additional pre-transfusion tests were performed on day 14, although no transfusion of RBC units was performed.

On day 19 after the first RBC transfusion, two RBC units were requested because the woman’s haemoglobin had decreased to 7.2 g/dL.

The antibody screening tests were still negative, according to our standard automated method. The same day of the request, she received one AB, CCDee standard packed red cell unit, with the Type & Screen procedure. The transfusion was interrupted 2.5 hours after being started because of a transfusion reaction: chills, lumbar pain and dark red urine.

The pre-transfusion and post-transfusion data indicate that there was no significant change in body temperature (pre-transfusion +37.2°C - post-transfusion +37.5°C) and minor modifications of blood pressure (pre-transfusion 130/65 - post-transfusion 140/85) and heart rate (pre-transfusion 78 beats per min - post-transfusion 88 beats per minute).

Dark red urine was still observed 24 hours after the reaction.

To determine the cause of the post-transfusion haemolysis, immediately after the reaction, the patient’s post-transfusion serum and plasma samples were inspected for evidence of haemolysis and compared with the plasma pre-transfusion sample, using the scale of values proposed by Elliot.

The post-transfusion samples were grossly haemolysed, with a dark red hue similar to haemolysis of 200 mL of RBC in 3,000 mL of plasma.

The post-transfusion biochemical values (Figure 1) revealed an increase in free plasma haemoglobin from 18.0 mg/dL to 260 mg/dL (reference value <5.5 mg/dL).

It was concluded that the patient suffered from the classic clinical symptoms of an acute haemolytic transfusion reaction (AHTTR), confirmed by concomitant biochemical changes.
Material and methods

Since other clinical or therapeutic causes of acute haemolysis, such as clerical error and mechanical haemolysis were excluded, an extensive immunohaematological work-up was carried out to search for the antibody responsible.

This work-up included the following tests.

1) Direct antiglobulin tube test (DAT): two volumes of reagent to one volume of a 3% red cell suspension, centrifugation, macroscopic reading, incubation at 20 °C for 5 minutes of all non-reactive tubes with polyspecific and anti-C3 antisera, centrifugation and macroscopic reading, addition to each negative tube of IgG-coated and C3-coated Cosmohb control cells (Immucor Inc., Norcross, GA, USA). We used undiluted and diluted (serial two-fold dilutions in saline) polyspecific and monospecific anti-human globulin reagents: anti-IgG+C3d from three manufacturers (Immucor; Diagnostics Scotland, Edinburgh, UK; Ortho-Clinical Diagnostics); anti-IgG from three manufacturers (Immucor; Diagnostics Scotland; Ortho-Clinical Diagnostics); anti-IgA and anti-IgM (Biotest AG, Frankfurt/Main, Germany). The rapid acid elution (ELU-KIT™ II, Immucor) was used for the recovery of antibody bound to red cells.

2) Indirect antiglobulin test (IAT), using plasma and serum and the standard method (Ortho BioVue, Ortho-Clinical Diagnostics) with polyspecific anti-human globulin, 50µL of LISS (Ortho® BLISS, Ortho-Clinical Diagnostics), 10µL of a 3% red cell suspension (from a screening panel - Surgiscreen Ortho-Clinical Diagnostics or red cells from selected blood donors), 40µL of plasma or serum, 15 minutes of incubation at 37 °C, centrifugation for 5 minutes and macroscopic reading; in addition, to demonstrate complement dependence of antibody in stored and aged sera, we also performed the IAT using fresh complement (from a pool of 50 blood donors of AB blood group) added to sera (one volume in three volumes of test sera).

3) Tube IAT (100µL of plasma or serum, 100µL of Gamma PeG™ additive, Immucor), one drop of a 3% red cell suspension, 15 minutes of incubation at 37 °C, four washes with saline, addition of two drops of monoclonal anti-IgG (Immucor), centrifugation for 1 minute and macroscopic reading.

4) Standard tube ficin-IAT using 100µL of plasma or serum, one drop of a 3% red cell suspension, 45 minutes of incubation at 37 °C, four washes with saline, addition of two drops of monoclonal anti-IgG (Immucor), centrifugation for 1 minute and macroscopic reading. The ficin-treated red cells (from the screening panel or from blood donors) were prepared using a commercial...
stabilised ficin solution (Ortho-Clinical Diagnostics) as suggested by the manufacturer.

5) Saline 20 °C: 100µL of plasma or serum, one drop of a 3% red cell suspension, 30 minutes of incubation at 20 °C, centrifugation for 1 minute and macroscopic reading.

6) Solid-phase IAT using a commercial panel for identification (Ready-ID®, Extend I and II, Immucor), 100µL of LISS additive (Capture®, LISS, Immucor), 50µL of plasma or serum, 20 minutes of incubation at 37 °C, six washes with saline, addition of one drop of indicator cells (Capture-R® Indicator Red Cells, Immucor), centrifugation and macroscopic reading.

Instead, for cross-matching (Capture-R® Select, Immucor), we prepared a 0.3-0.5% suspension of well washed red blood cells in saline, 50µL of the red blood cell suspension, centrifuged the strip, carried out six washes with saline, and proceeded with LISS and plasma or serum as described for identification.

7) Erythrocytes Magnetized® technology®3 (Screenlys, Diagast, France), which uses IgG-coated plates. To prevent the neutralisation of anti-IgG 60µL of low-ionic and diluent (Nano-lys and Screen Diluent, Diagast) solutions were dispensed before the addition of samples and RBC (12µL plasma, 15µL of a 1% three-cell panel, Hemascreen, Diagast). After incubation, without any washing/centrifugation, magnetisation was performed and the plates were placed on a magnetic workstation (FreeslyNano, Diagast) that allowed the adherence of sensitised-magnetic RBC.

8) Typing of the red cells of the patient and transfused units was performed using the standard agglutination tube methods.

Results
During our investigations, at the time of collecting samples, no haemodilution was performed in the patient by infusion of saline or other liquid.

Table I - Results of serological investigations

<table>
<thead>
<tr>
<th>Time</th>
<th>IAT±volume (standard screening)</th>
<th>Cross-match</th>
<th>DAT†-column</th>
<th>Identification of antibodies in serum</th>
<th>No. of transfused RBC units</th>
<th>AHTR</th>
<th>Status in IAT test</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Negative</td>
<td>No, T&amp;S</td>
<td>Negative</td>
<td>Nd</td>
<td>1 (Packed RBC)</td>
<td>No</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>Negative</td>
<td>No</td>
<td>Negative</td>
<td>anti-Bc only in SP and EMT(9)</td>
<td></td>
<td></td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Day 19 Pre-transfusion</td>
<td>Negative</td>
<td>No, T&amp;S</td>
<td>Negative</td>
<td>anti-Bc only in SP and EMT(9)</td>
<td>1 (Packed RBC)</td>
<td>Yes</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Day 19 Post-transfusion</td>
<td>Negative</td>
<td>No, T&amp;S</td>
<td>Negative</td>
<td>anti-Bc only in SP and EMT(9)</td>
<td></td>
<td></td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Day 20 Post-transfusion</td>
<td>Negative</td>
<td>No</td>
<td>Negative</td>
<td>anti-Bc only in SP and EMT(9)</td>
<td></td>
<td></td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Day 21 Post-transfusion</td>
<td>Weak (score ±)</td>
<td>No</td>
<td>Negative</td>
<td>anti-Bc only in SP column, only with 2 out of 4 Jk(a+b+) red cell suspensions tested</td>
<td></td>
<td></td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Day 25 Post-transfusion</td>
<td>Negative</td>
<td>No</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Day 32 Post-transfusion</td>
<td>Negative</td>
<td>No</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

(*) IAT: indirect antiglobulin test;  
(**) DAT: direct antiglobulin test;  
†) SP: solid-phase method;  
‡) EMT: erythrocyte magnetised technology;  
($) AHTR: Acute Hemolytic Transfusion Reaction.
The results of our investigation are reported in table I. An anti-Jka was detected only by the solid-phase method and Erythrocytes Magnetized® technology in the sample of day 14 (after the first transfusion), while all other methods gave negative results. No differences were detected between serum and EDTA plasma. Two out of four cells Jk(a+b-) show weak positive results for the presence of anti-Jka by the column agglutination method, using the plasma sample of day 21 (2 days after the reaction).

These two cells had a double dose expression of the target antigen. This result was not confirmed by further investigations on the samples drawn on day 25 and day 32 after the first transfusion. The tube DAT was negative for all samples tested. Despite this, we performed an elution from pre-transfusion and post-reaction blood samples (day 19) and tested them by tube PEG-IAT and by the solid phase method. The anti-Jka was detected in the post-transfusion eluate, using only the solid phase method.

No antibodies were detected in any samples by microcolumn agglutination, using serum with fresh complement added. The patient's RBC phenotype, determined on pre-transfusion and on all post-transfusion samples, was A, B, CcDee, Jk(a-b+), Fy(a+b+), M+N+S+s+.

Samples from the first RBC unit transfused and the unit involved in the reaction were available and typed as Jk(a+b+) and Jk(a+b-), respectively.

The antibody screening test was negative in both blood donors using the solid-phase method and positive results in cross-matches were detected only in solid-phase, using the pre and post-reaction samples (day 19), while results were negative in solid-phase using selected AB, CcDee, Jk(a-b+) units.

After our investigations, the patient recovered and needed no further transfusions.

Discussion

After the discovery of the first anti-Jka, many cases involving Kidd system antibodies have been reported and a substantial number have been implicated in AHTR. Kidd antibodies are usually IgG or a combination of IgG and IgM; pure IgM examples are rare. IgM Kidd antibodies can bind the complement and are consequently detected only by broad spectrum antiglobulin reagents, containing an anti-complement component.

It has been generally considered that the major antibody component of Kidd antibodies must be IgG with complement-fixing ability. Currently, guidelines for pre-transfusion testing indicate that antiglobulin reagents with a potent anti-IgG can effectively detect such antibodies, without the need of an anti-complement component.

This has encouraged Blood Transfusion Centres to use techniques which compromise the detection of the complement, on the basis that the antibodies will be detected by the anti-IgG component of the antiglobulin reagent. We report a case in which the IgG component of an alloantibody-Jka was demonstrated only in solid-phase and by Erythrocytes Magnetized® technology, but not detected in the patient's plasma or serum, when tested by microcolumn agglutination with broad spectrum antiglobulin or in tubes with anti-IgG antiglobulin reagent.

The antibody was the cause of an AHTR associated with the transfusion of Jk(a+) blood units. The use of plasma rather than serum was not the cause of false negative results in the initial screening tests, although the column agglutination tests (using gel technology) can compromise the detection of Kidd antibodies.

This problem may sometimes be associated with shear forces.

Because the primary immune response could have already occurred with a previous pregnancy, the first transfusion of random Jk(a+b+) could have caused a secondary immune response, that resulted in the haemolysis of the second Jk(a+b-) transfused unit.

As recently reported by the results of a large retrospective multicentre study, we determined that the anti-Jka was the cause of a secondary response in a short interval of time after transfusion (14 days after the first transfusion); the level of antibody was such that serological detection was only possible using the most sensitive technique.

This same circumstance was reported by Callahan et al.: the Authors described an anti-Jkb, detected only in solid-phase and not by PEG, gel or LISS techniques, which caused a delayed haemolytic transfusion reaction in a patient affected by sickle cell disease.

Naturally occurring anti-Jka antibodies, detectable only in solid-phase, were previously reported by Ramsey et al. and the use of manual polybrene was the method of choice for the detection of weak anti-Jka. During the investigations, 2 days after our patient's reaction, the antibody increased to levels detectable by our standard method and then immediately declined to a point at which antibody was no longer detectable in...
This case demonstrates one example of a haemolytic transfusion reaction due to anti-Jk$^\alpha$, which was not detectable with a highly sensitive routine test method (microcolumn-agglutination).

Our data document the well-known difficulties in the detection of Kidd antibodies and highlight the importance of using additional sensitive techniques and multiple methods, particularly in cases of haemolytic transfusion reactions.

In this case, significant haemolysis caused by anti-Jk$^\alpha$ was detected only in solid-phase and by Erythrocytes Magnetized® technology and not by column or liquid-tube technologies.

References
2) American Red Cross Immunohematology. Methods and Procedures. 1993 American Red Cross, Rockville.
Dear Editor,

the main priority of voluntary blood donor associations is to spread the practice of donating blood and its components, thus guaranteeing self-sufficiency in these products; this can be achieved principally through increasing the number of organised and periodic donors.

According to some statistical estimates, the population of over 60-year olds will increase from 998,142 in 2006 to 1,611,447 in 2046 (+613,305), while the population between 18 and 60 years old will decrease from 5,418,693 to 4,045,176 (-1,373,517). On the basis of these demographic projections, we considered a more significant involvement of people from other parts of the world, with different cultures, religions and ethnicities.

In our opinion, the pathway of intercultural harmony, integration and interactions should undoubtedly start from aspects of health care, which must be based on scientific analysis and careful evaluation of the existing literature, but must have a strong social value.

Although the opinions above are not exhaustive and worthy of further analysis, they do stimulate reflection and allow us to make some considerations on a subject, that is perhaps a novelty only for us, and examine it without preconceptions or prejudices. Where the development of a culture of donation passes through the awareness and the responsible involvement of the individual, health care issues constitute an important but not unique criterion, that must guide us.

For example, if we take risk behaviours, as we understand them, these can have completely different facets depending on the person’s culture and/or religion, which are not necessarily linked to those of the country of origin. This problem is also clearly present in already existing donors.

Finally, participation in the development of voluntary activities and unpaid, periodic donations is a significant and concrete form of interaction, efficiently promoted by voluntary blood donor associations.

On this background and based on a predominantly anthropological approach, we set up the “Project to promote blood donation in a multicultural society”. The stages of this project were as follows:

- the establishment of a scientific committee (see appendix), co-ordinated by the author of this letter and supervised by the anthropologist Annamaria Fantauzzi, with the active participation of associations of the Latin-American, Romanian, Moroccan and Sikh communities, as well as the Union of Islamic Communities in Italy (UCOI);
- the first meeting, held in January of this year, during which the following aims and strategies were decided;
- organisation of a meeting: “Blood donation in a multiethnic society”, held on March 17th in Milan. This meeting was aimed at representatives of groups and associations of foreigners and voluntary blood donor associations, community leaders and/or reference figures for foreign citizens. Some of these community figures were speakers at the meeting. There were 119 participants [52 from the Italian Association of Voluntary Blood Donors (AVIS), 10 from the Policlinico Hospital in Milan, and 57 from the different communities involved, including the Philippine community]. The meeting was followed by a multiethnic buffet. The following aims have been defined for this project.

1. Promote the culture of solidarity and donation.
2. Promote the practice of donating blood within various communities of foreigners, while respecting their different cultures and religions.
3. Spread clear information and indications on how to get to know AVIS and other donor associations and their actions in local, national and international settings.
4. Set up and promote activities aimed at creating a
network between the people involved in order to:

a) exchange information, experiences, strategies and
to enhance awareness;
b) facilitate involvement and meetings;
c) promote joining AVIS and other associations;
d) participate in meetings.

5. Create the instruments for carrying out campaigns to
increase awareness within reference points for foreign
families and in meeting places.

6. Facilitate the availability of information about rare blood
groups.

7. Create networks for the development of cultural and
scientific exchanges with the countries of origin of the
foreign communities (for example, reciprocal visits,
exchange of informative material, relations between rare
blood group banks, invitations to particularly significant
moments of our association, such as assemblies,
meetings, exhibitions and celebrations).

At the end of the meeting, we set up a mixed working
group, that will work to produce material, in different
languages, to promote blood donation and to organise
various manifestations, one of which could take place
concurrently with the World Blood Donor Day on June
14th, 2007.

Appendix

Project leader
- Lombardy and Piedmont Regional Sections of AVIS,
in collaboration with the Lombardy Regional Bank of
Rare Blood Groups and the Association of Donors of the
Policlinico Hospital, Milan.

Scientific Committee
- Sebastian Asaftei, Orthodox Church (Turin)
- Mohamed Boussouita, AMECE Association - Morocco
- Giorgio Diako, President of AVIS, Piedmont Region
- Anna Maria Fantini, ethnologist, La Sapienza
University of Rome - EHESS Paris, Head of the
Observatory on the culture of blood donation by
immigrants of the National AVIS:
- Stelian Ionescu, Association of Romanians in Italy
(Milan)
- Yassin Lofoum, Young Muslims in Italy - UCICR
- Vincenzo Saturni, President of AVIS, Lombardy Region
(coordinator)
- Sukhdev Singh, Sikh community (Cremona)
- Maria Pilar Yenque, Latin America Association
- Giorgio Marmiroli, Blood Donors Association of the
Policlinico Hospital (Milan)

AVIS representatives
- Igino Arboatti, Health Care Director - AVIS, Piedmont
Region
- Giampietro Brusola, Health Care Director - AVIS,
Lombardy Region
- Alda Ciarlani, Councillor - AVIS, Lombardy Region –
School representative
- Claudio Crocco, Councillor - AVIS, Lombardy Region
- Youth policies representative
- Sandro Fisso, Administrator - AVIS, Piedmont Region
- Felice Majo, Vice-President - AVIS, Lombardy Region
- Luisella Pavesi, Councillor - AVIS, Piedmont Region –
Head of Training.
- Tiziana Tacchini, Vice-President - AVIS, Piedmont
Region

Representative of the Regional Rare Blood Groups
Bank:
- Fernanda Morelati, Chief of the Regional Rare Blood
Groups Bank
- Maurizio Marconi, Director of SIMT, Policlinico
Hospital, Milan

Representative of the Blood Donors’ Association of
the Policlinico Hospital, Milan
- Giorgio Marinari, Vice-President
- Giovanna Cremonesi, Health Care Director

Organising Secretariat
- Licia Caceddu - Danila Fiume, AVIS, Lombardy Region
Duffy and Kidd blood group antigens: minor histocompatibility antigens involved in renal allograft rejection?
Transfusion 2007; 47: 28-40

There is increasing evidence that minor histocompatibility antigens play a role in the graft rejection process. Their characterisation is, therefore, of importance, but very complex and they are still poorly defined. Lerut and colleagues from the University of Leuven (Belgium), from the University of Paris (France), and from the National Institute of Blood Transfusion in Paris investigated whether the Duffy (FY) and Kidd (JK) blood groups antigens could play a role as minor histocompatibility antigens in renal allograft rejection. These polymorphic and immunogenic antigens are widely distributed in human organs, are expressed and functional in the kidney, and combine particular proprieties, strongly suggesting that they could indeed act as minor histocompatibility antigens in renal allograft rejection. Three hundred and seventy cadaver renal transplants (342 first, 28 non-first transplants), performed in the Renal Transplant Programme at the Catholic University of Leuven between January 1, 1991 and March 31, 1995, with an immunosuppressive regimen containing cyclosporine A and corticosteroids, were studied retrospectively; the recipients were followed-up for more than 7 years after transplantation. All donors and recipients were Caucasian. All relevant data (demographic, clinical, biological and immunohaematological) were prospectively collected and entered in the University of Leuven renal transplant database. When included in the programme, each recipient received transfusion with two units of concentrated, non-leucodepleted red cells. The following characteristics were studied: age and sex of donor and recipient; donor/recipient sex mismatch; number of HLAA, -B and DR mismatches; cold and warm ischaemia time; mean (mg/kg/day) dose and total dose (mean dose x number of days administered/10^3) of cyclosporine A and its level at 1 and 5 years after transplantation; maximal percent of panel reactive antibodies; presence of anti-FY and anti-JK antibodies at day 0 and after transplantation. FY and JK polymorphisms were identified in all donor/recipient pairs by real time polymerase chain reaction (genotyping) and in all pairs the matching or mismatching status was defined for both systems. All biopsies were reviewed and historical screening results for FY and JK alloantibodies and graft survival were retrospectively analysed. The results strongly suggest an immunological role for the FY polymorphism in renal transplantation. It was, in fact, observed that FY mismatched grafts had significantly more chronic lesions than FY matched grafts, therefore supporting a minor histocompatibility status for FY. HLA-DR11 was more frequent in both recipients and donors of FY mismatched pairs without chronic allograft nephropathy, suggesting a protective effect of this molecule.

The results regarding JK polymorphisms are less clear, although JK mismatched grafts had higher scores for interstitial inflammation than JK matched grafts. FY and JK matching had no influence on graft survival. As well known, most of blood group antigens are not solely confined to erythrocytes but are widely expressed through the human organs, and, therefore, the term "histo-blood group antigens" has been correctly introduced. In summary, the observations and the results of this important retrospective study strongly support the idea that FY could play a role as a minor histocompatibility antigen. Although histological differences were detected between matched and mismatched JK, the data were less strong than those for the FY system.
A prospective study on the prevalence and risk factors for neonatal thrombocytopenia and platelet alloimmunization among 9,332 unselected Brazilian newborns.


The article presents a screening study on the prevalence of neonatal alloimmune thrombocytopenia (NAIT) in Brazilian newborns, a population characterised by admixture of indigenous, African, and Caucasian subjects. The study was performed at the State University of Campinas (Brazil), with the collaboration of colleagues from Germany and the United States. NAIT develops as a result of an interaction between human platelet antigen (HPA) HLA allotypes and the ethnic background of the population. NAIT is present in 0.5% to 0.9% of unselected Caucasian newborns. Its prevalence in other populations is unknown. A series of studies among Caucasians revealed that 80% of NAIT results from disparity in the tetradic HPA-1 system: approximately 2% of Caucasians lack the common HPA-1a allele and are at risk of NAIT. However, the disease occurs in only 10% of these people and is strongly associated with the maternal HLA-DRw52a type. The HPA-5 system is also commonly involved in NAIT among Caucasians, but presents with milder clinical pictures, compared with the HPA-1 mediated disease.

The study was carried out, prospectively, over a 3-year period, to determine the prevalence and the causes of thrombocytopenia in cord blood samples. The platelet count was performed on cord blood, using a standard automatic blood cell counter. Genotyping for HPA-1, -2, -3, and -5 was performed (in pairs of thrombocytopenic neonates) by polymerase chain reaction (PCR)-restriction fragment length polymorphism, and for HPA-4 by PCR with allele-specific primers. Sera of all mothers with genotypic mismatch from each group were tested for the presence of anti-HPA antibodies, using the monoclonal antibody immobilisation of platelet antigen (MAIPA) technique. Thrombocytopenia (defined as a platelet count below 100 x 10^9/L) was detected in 142 out of 9,332 newborns studied (1.5%). The majority of thrombocytopenic neonates (128 cases, 90.1%) had platelet counts between 50-100 x 10^9/L; moderate thrombocytopenia (<50 x 10^9/L) was detected in 12 (8.5%) cases, and severe thrombocytopenia (<20 x 10^9/L) in only two (1.4%) cases. Bleeding was observed in 17 (12%) of the 142 thrombocytopenic newborns and underlying diseases were present in 48% of these 142 neonates. Maternal-newborn HPA genotype mismatch occurred in 50% of gestations, but did not predict the risk of thrombocytopenia. Nevertheless, it is notable that mismatched genotypes for HPA-5 were slightly increased in the thrombocytopenic group. Anti-HPA-5b antibodies were observed in 0.05% of unselected pregnancies, but in 12% of mothers of neonates with thrombocytopenia and mismatched genotype (51 cases).

In conclusion, thrombocytopenia is relatively common among unselected Brazilian newborns, with a similar prevalence to that previously described in other populations. A notable feature of NAIT in this population was the involvement, in most cases, of the HPA-5 system, reinforcing the need for the development of different approaches to NAIT in distinct ethnic groups.


Given the large success of immunoprophylaxis of haemolytic disease of the newborn (HDN), due to anti-D incompatibility, the main source of anti-D immunoglobulins for further prevention, namely immunised women, has been almost exhausted. Deliberately immunised men have also been used as a source for the production of anti-D immunoglobulins, but, for safety reasons, are not an ideal source. For the above-mentioned reasons, an unlimited and non-plasma-derived source of antibodies for D-prophylaxis is needed. Recombinant anti-D IgG has been produced by several groups, but all is still in an experimental phase of studies and it is not known whether these antibodies have the potential to replace the polyclonal Rh prophylaxis.

Recombinant human IgG (IgG1, IgG2, IgG3, IgG4) and recombinant IgA (IgA1 and IgA2), with the same variable regions, were expressed in Chinese hamster ovary cells. Their effector functions were assessed by an antibody-dependent cell-mediated cytotoxicity (ADCC), and by a chemiluminescence (CL) method for the detection of "respiratory burst" (a rapid release of reactive oxygen species from different types of immune cells). Their effector functions were assessed by an antibody-dependent cell-mediated cytotoxicity (ADCC), and by a chemiluminescence (CL) method for the detection of "respiratory burst" (a rapid release of reactive oxygen species from different types of immune cells).

In the ADCC assay, IgG1, IgG3, and IgA1 gave the best results and were as active as currently used prophylactic anti-D antibodies. IgG4 and IgA2 were moderately active, whereas IgG2 were not active, at all.

In CL, IgG1 and IgG3 were active, but much less so than a currently used prophylactic polyclonal anti-D. Also in CL, IgG2 were not active. A mixture of recombinant IgG1 (rIgG1) and rIgG3 did, however, show a synergistic effect: no significant difference in the ability to mediate a CL
response, compared to the polyclonal anti-D, was shown to be effective in differentiating between mixtures containing 75% rIgG1 and 25% rIgG3 and those containing 50% rIgG1 and rIgG3. Mixtures of rIgA1 and either rIgG1 or rIgG3 showed no synergic effect.

The inability of rIgG2 anti-D to mediate a response in both assays was ascribed to the topography, surface density, and restricted mobility of the D antigen. Anti-D is bound near the lipid bilayer, surrounded by glycocalyx, and this may sterically hinder the access of the relatively inflexible rIgG2 anti-D, but not that of rIgG1 or rIgG3 anti-D.

In conclusion, in vitro functional studies on recombinant anti-D antibodies (rIgG and rIgA), performed at Copenhagen University Hospital, showed that in the ADCC and CL assays no single antibody was comparable to a currently used prophylactic polyclonal anti-D in the ability to induce a respiratory burst and to promote lysis of red blood cells. A combination of rIgG1 and rIgG3 did, however, demonstrate similar activity in CL and ADCC assays as polyclonal anti-D, and could be of value in future immunoprophylaxis for prevention of HDN due to D.


In countries with large migrant populations, the supply of compatible blood for transfusion in recipients from distant geographic regions may be a problem. Indeed, red blood cell (RBC) polymorphisms can lead to significant phenotypic discrepancies between patients from migrant communities and the local donor pool, and some populations can be considered as high-risk groups for alloimmunisation. This is especially true for sub-Saharan Africans, living in countries with predominant Caucasian (European) populations. Haematological hereditary diseases, such as sickle cell anaemia, and traditional cultural values that discourage blood donation further complicate the problem. Marseilles, in France, where this study was performed, has shown a strong capacity for integrating immigrant populations. While migrants have traditionally been from bordering countries (Italy, Spain, North Africa), recent immigration has been more diverse, with a growing number of people from sub-Saharan Africa, Asia, and East Europe. Already numbering 70,000 persons, one of the fastest growing and largest minorities in the south of France, are immigrants from the Comoros Islands (located between northern Madagascar and northern Mozambique). It has been found that Comorian immigrants have a typical sub-Saharan African phenotype, characterised by the absence of a high frequency antigen in the Duffy system (F3 and F5), by the Fy(a-b-) phenotype (also in the Duffy system), U-" or S-" in the MNS system, and by the presence of i"(Ch) allele in the Rhesus system.

The purpose of the report was to present the method used in a drive to promote blood collection in the community formed by Comorian immigrants living in Marseilles. With a culturally adapted message, developed on an anthropologic approach, and working in close collaboration with scientific and medical members of the Comorian community, this population has been sensitised to blood donation. An association of voluntary blood donors of Comorian origin was also founded. Involvement of religious and political leaders was essential to support the intervention and to dispel any ambiguity about objections to blood donation.

As result, a targeted collection of specific sub-Saharan blood phenotypes was achieved, recruiting mostly second-generation donors. At first, a total of 92 people of Comorian origin volunteered to donate blood. Based on the medical pre-donation interview, only 59 individuals were selected for collection: 33 candidate donors were excluded, most because of a history of malaria or exposure to infectious disease. The first collection was effective in providing specific red blood cell phenotypes, present in 7 donors (nearly 12%), a very high percentage compared with only 0.56% in the previously existing donor pool. Subsequently, it was decided to promote regular blood donation by addressing people directly towards the principal Blood Transfusion Centre in Marseilles.

In conclusion, the approach led to an increase of new, regular and voluntary Comorian blood donors. This method could also be applied to improving donation of peripheral blood stem cells or organs from the same population.

Wagner SJ, Eder AF A model to predict the improvement of automated blood culture bacterial detection by doubling platelet sample volume Transfusion 2007; 47: 430-33.

In the USA, blood banks initiated automated cultures for detecting bacteria in apheresis platelet concentrates (PC), in response to the voluntary standards of the College of American Pathologists and American Association of Blood Banks (AABB). Some bacterial infections have been transmitted through apheresis PC. Results from testing 1,277,508 platelet apheresis collections from March 2004 to February 2006 were reported by the American Red Cross (ARC). The results were: a true culture-positive rate of 0.56% in the previously existing donor pool. Subsequently, a true culture-positive rate of 0.56% in the previously existing donor pool. Subsequently,
approximately 1 in 5,400; a confirmed false-positive rate of approximately 1 in 2,900 units, and an unconfirmed and indeterminate rate of approximately 1 in 12,900. During this period of time there were, however, 17 septic transfusion reactions from PC with false-negative results, including two fatalities. Thirteen of these 17 cases involved *Staphylococcus* species, which are known to be slow-growing organisms, that exhibit lag times when deliberately inoculated into platelet components. In this report, Wagner and Eder from the ARC Blood Department (Rockville, MD) and the ARC Blood Services (Washington, DC) explored the impact of doubling the sample volume (from 4 to 8 mL) for culture detection of slow-growing bacterial organisms. Bacterial growth was modelled by varying the initial inoculum, doubling time, and lag time. The numbers of organisms present at a 24-hour sampling time were calculated. Limitation in sampling units with low bacterial load and the possibility of self-sterilisation of units during processing time complicated the direct assessment of bacterial growth in the spiking studies. The Authors, consequently, used a mathematic model coupled with Poisson analysis to predict the improvement of bacterial detection by doubling the sample volume. From each inoculum, the percentage of improved detection was defined as delta, i.e. the difference between the percentage of detection in 8- and 4-mL samples. The results suggest that: 1) the maximum improvement of detection was independent of bacterial doubling time or lag time; 2) the maximum improvement occurred with inocula <40 colony-forming units (CFU) per single apheresis PC; 3) doubling the sample volume improved bacterial detection by only 25% or less. Other approaches, that may have a greater impact on improving the detection of *Staphylococcus* organisms in PC, include delaying the time of sampling for bacterial culture beyond 24 to 36 hours (which would be impractical for many Transfusion Centres for maintaining blood component availability) and further development and/or implementation of rapid bacterial tests, administered just before transfusion, which are capable of detecting bacterial levels of less than 10^4 CFU/mL.


Transfusion 2007; 47: 599-607.

Transfusion-related acute lung injury (TRALI) is characterised by pulmonary oedema and hypoxaemia within 6 hours after a transfusion in the absence of other causes of acute lung injury or circulatory overload, and is, now, the leading cause of transfusion-related death reported, in the USA, to the Food and Drugs Administration (FDA). It is likely that the overall morbidity associated with TRALI exceeds that suggested by fatality reports, because the majority of TRALI cases are non-fatal and even fatalities are underreported. American Red Cross (ARC) surveillance data on TRALI fatalities were analysed to evaluate the association with components from donors with white blood cell (WBC) antibodies and to examine the potential impact of the selective transfusion of plasma from male donors. The Donor and Recipient Complications Program (DRCP), maintained by the ARC, appears well suited to this purpose. All transfusion reactions investigated for TRALI between January 1, 2003, and December 31, 2005, were identified in the DRCP database, and all fatalities were classified, by three physicians, as probable TRALI or of unrelated aetiology. Each case was given a probability score: P1 = insufficient information; P2 = unlikely to represent TRALI; P3 = clinical presentation suggestive of TRALI but an aetiology other than TRALI present; P4 = clinical presentation compatible with TRALI but case not typical; P5 = consistent with TRALI but a WBC antibody-containing component not identified; P6 = consistent with TRALI and a WBC antibody-containing component identified.

A total of 550 reports of suspected TRALI, including 72 fatalities, were retrospectively investigated. The number of reports increased each year and the rate varied by geographic region. The review of fatalities revealed 38 cases of probable TRALI (P5, P6), the majority (24 out of 38, 63%) after plasma transfusions. A female, WBC antibody-positive donor was involved in 27 out of 38 (71%) cases, and in 18 out of 24 (75%) cases involving plasma transfusions. Female antibody-positive donors were more likely to be associated with probable TRALI than with unrelated cases (p = 0.001). The rate of probable TRALI among recipient fatalities was higher for plasma components or apheresis platelet concentrates than for red blood cell concentrates. As regards the proportion of male to female donors of distributed blood components, males contributed 64.5% and 52% of apheresis platelet concentrates and plasma components, respectively, in 2005.

In conclusion, plasma components from female donors with WBC antibodies were responsible for the majority of TRALI fatalities. Proliferative measures to limit transfusion of WBC antibody-containing plasma components may lower the incidence of this type of fatality.
A revised classification scheme for acute transfusion reactions

The classification of transfusion reactions is summarised in the AABB Technical Manual, which serves as a standard reference for Transfusion Medicine Services. In the AABB classification the acute reactions, occurring within 24 hours after transfusion, are divided by presumed aetiology into immunological and non-immunological subtypes. The immunological types include the following reactions: haemytic, fever and/or chill non-haemytic, urticarial, anaphylactic and transfusion-related acute lung injury (TRALI); the non-immunological reactions include: hypotension associated with ACE inhibition, circulatory overload, non-immune haemolysis, air embolism, hypocalcaemia, hypothermia and transfusion-associated sepsis. Although this system adequately describes the general features associated with the various types of acute transfusion reactions, it was not designed to provide strict criteria for diagnosis and classification. Consequently, its use to classify individual reactions can result in significant inter- and intra-observer variability, leading to unreliable comparisons of research results between different Centres and between studies from the same Centre.

Sanders and colleagues from the Department of Oncology at St Jude Children’s Hospital in Memphis (TN, USA) reviewed blood bank and medical records to identify all suspected transfusion reactions that occurred in their Institute from January 1, 1996 until December 31, 2003. Initially classified according to the AABB system, each reaction was then reclassified with a revised model, which refines and clarifies various categories, and adds severity grades in the format of the Common Terminology Criteria for Adverse Events (CTCAE), a system created by USA National Cancer Institute, to standardise the reporting of adverse events in cancer therapy. The revised system includes the following categories: febrile asymptomatic, allergic, inflammatory, mixed allergic and inflammatory, TRALI, hypotension, haemolysis (immune or non-immune), sepsis, circulatory overload. It also incorporates a severity grade (mild, moderate, severe, life-threatening, fatal). Further groups of the revised system consider the likelihood of relation to transfusion: unrelated, unlikely, possible, probable, definite. All blood components administered to the patients were irradiated and leucoreduced; platelet concentrates were all single donors apheresis units. During the 8-year study period, 627 suspected transfusion reactions were reported and 595 of these had complete records suitable for analysis. Three hundred and seventy-two occurred with transfusion of platelet concentrates, 213 in red blood cell transfusions, 5 in fresh-frozen plasma transfusions and 3 in transfusions of granulocyte concentrates. In additional two cases, patient receiving both platelet concentrates and erythrocyte concentrates within a short interval and the blood component that elicited the reaction could not be clearly distinguished.

A comparison of the classification approaches applied to these 595 transfusion reactions showed clear advantages of the revised system. Of 128 reactions classified by AABB criteria as inconclusive (mixture of reaction types or otherwise qualified), all but 5 could be classified into discrete categories in the new scheme. The revised system introduces more specific criteria for certain reactions and permits the grading of severity and attribution (likelihood that the adverse event is related to transfusion), leading to more thorough and more flexible categorisation of transfusion reactions.

Antibodies to a novel antigen in acute hepatitis C virus infection

Hepatitis C virus (HCV) is the aetiological agent of a liver infection that affects, worldwide, about 170 million people. Commercially licensed HCV enzyme immunoassays (EIA), which are used to screen for HCV exposure, use recombinant proteins, presenting linear epitopes. It is, however, recognized that conformational antigens may play an important role in the immunobiology of acute HCV infection and may enable earlier antibody detection. In the late 1990s, a new donor-screening method, nucleic-acid amplification technology (NAT), was introduced: this technology enables the detection of HCV RNA positive, but antibody non-reactive, infected units of blood during routine donor screening. Very rarely, some HCV-infected people apparently fail to produce antibodies, but most samples of this type (RNA positive/antibody negative) simply represent very early HCV infection.

Tobler and co-workers, from the Blood System Research Institute in San Francisco (CA, USA), evaluated early antibody production, using three new EIAs, containing antigenic proteins not present in licensed assays. The first new EIA contains two proteins: a conformational antigen that retains both protease and helicase enzymatic activities (NS3/4a), and a single multiple-epitope fusion antigen (MEFA 7.1). MEFA 7.1 represents an enhanced antigen, incorporating known major epitopes of HCV protein. The
second EIA used the Fv (frameshift) and core proteins of hepatitis virus as the anti-HCV detection antigens. The third research EIA used E1/E2 protein generated in Chinese hamster ovary (CHO) cells as the anti-HCV detection antigen. Forty-two RNA positive/EIA 3.0 (latest immunoassay licensed) negative samples, including two persistently serosilent cases, were used to evaluate these research EIA. Fifty-four EIA 3.0 negative/RNA positive/antibody positive specimens were included, as controls. Only the MEFA 7.1 NS3/4a was positive in seven (17%) of the 42 HCV RNA positive samples, in all three positive controls but in none of 54 EIA 3.0 negative/RNA negative controls. Thirty-five specimens, including the two serosilent case were negative. Notably, six out of the seven (86%) MEFA 7.1 NS3/4a positive samples had evidence of active hepatitis (ALT >210 IU/L). Conversely, all 42 NAT yield specimens, including the serosilent cases, were non-reactive in the research EIA assays, using both the Fv/ore protein combination and the CHO E1/E2 antigens. All 54 negative specimens used as controls were non-reactive with the three research EIAs, while the RNA+/antibody controls were positive. 

In conclusion, a novel research EIA, using conformational and enhanced linear epitopes (NS3/4a and MEFA 7.1 NS3/4a) to detect HCV antibodies in 17% of viraemic donations missed by the HCV 3.0 EIA. This research EIA appears to detect HCV antibodies closer to the initiation of acute hepatitis. Given that the average RNA-positive/antibody negative window period is ~56.4 days, this 17% yield would translate into a ~10 day earlier detection of HCV antibodies.


The Duffy (FY) blood group antigens have been known, for many years, to be a receptor for malaria parasites Plasmodium vivax and Plasmodium knowlesi. More recently, the Duffy protein was found to be a receptor for various classes of chemokines and was renamed DARC (Duffy antigen receptor for chemokines), a seven transmembrane glycoprotein present on erythroid and non-erythroid tissues. FY is a clinically significant blood group system, involved in haemolytic transfusion reactions and in haemolytic disease of the newborn. The system is controlled by four major alleles: FY*A and FY*B, the Caucasian common alleles, encoding for FY* and FY* antigens; FY*A allele responsible for reduced expression of FY* antigen; and FY*Fy, a silent gene. Homozygosity for FY*Fy gives rise to the Fy(a-b) phenotype, common in Black people but extremely rare in Caucasians. In Fy(a-b) Black individuals the FY*Fy gene is a T46C mutation in the promoter region, which abolishes erythroid expression of the FY gene, that is, in contrast, normally expressed in non-erythroid tissues. The FY*X allele, found more frequently among Caucasians, is caused by a C286T mutation in the FY*R gene, resulting in an Arg to Cys substitution at position 89 of the Duffy protein.

Despite the recent development of a real-time fluorescent polymerase chain reaction (PCR) method for FY genotyping, FY*X genotyping has not been described by this method. The study by Ansart-Pirenne et al. focused on the real-time FY*X genotype development, associated with a complete, one-step real-time FY genotyping, based on fluorescence resonance energy transfer (FRET) technology. The study was carried out at the Institut National de la Transfusion Sanguine in Paris (France).

Two series of blood samples were studied: i) 72 blood specimens from Fy(a-b-) Caucasian blood donors, studied by real-time PCR only; ii) 47 blood samples from Caucasians and Black patients referred to the Laboratory of the Institute, and studied both by real-time PCR and by PCR-restriction fragment length polymorphism (RFLP). The Duffy phenotypes were determined by an agglutination method using an indirect antiglobulin gel test with anti-Fy and anti-Fy* human polyclonal sera. When necessary, weak expression of FY* was confirmed by adsorption/elution studies.

Two mutations (C286T and A319G) were identified in the FY*X gene: only the C286T single nucleotide polymorphism (SNP) is associated with the weak FY* allele. The frequency of FY*X allele in the Fy(a-b-) donors was 0.014, identical to the frequency previously described (0.015). In 68 out of 72 donors the genotype was FY*A/X and in two FY*A/Fy. With the Caucasian and Black patients, a complete correlation between PCR-RFLP and real-time PCR was found, whatever the allele combination tested. When the known phenotype was not correlated to FY*X genotype, the presence of the FY* allele was always confirmed by adsorption/elution studies.

In conclusion, the real-time technology method is rapid and accurate for FY genotyping and able to detect the FY*X gene in all the allele combinations studied. The detection of the FY*X allele is useful for determining the

Blood Transfus 2007; 5: xx-xx
Fy* antigen status both in blood donors and in recipients, considering the transfusion use of blood units and the preparation of panels for antibody screening.

Vamvakas EC
White-blood-cell-containing allogeneic blood transfusion and postoperative infections or mortality: an updated meta-analysis
Vox Sang 2007; 92: 224-32

Vamvakas, one of the researchers most interested in transfusion-related immunomodulatory (TRIM) effects of allogeneic blood transfusion (ABT), tried to undertake a final meta-analysis that should generate conclusions, based on all randomised controlled trials (RCT) published on the matter. In fact, no further RCT are presently either planned or underway. The purported TRIM effects of ABT remain debated and elusive, but most evidence from animal models suggests that TRIM is mediated by white blood cells (WBC). The donor’s WBC may either directly down-regulate recipient immune functions or indirectly mediate the TRIM effects by releasing soluble mediators into the supernatant fluid of red blood cells (RBC), during storage. As mentioned above, new studies are not underway and, given that most transfusions currently administered in North America and in Western Europe are WBC-reduced, it will be difficult (if not impossible) to undertake further RCT comparing recipients of non WBC-reduced vs. WBC-reduced RBC.

RCT reporting on the association of ABT with post-operative infections and/or short-term (up to 3 months after transfusion) all-cause mortality were retrieved using a computerised (MedLine) search of the English literature, a manual search of the bibliographies of all pertinent articles, and a manual search of abstracts appearing in the supplements of scientific journals (of transfusion medicine, haematology, anaesthesiology/critical care). Twelve RCT reporting on infections and 11 RCT reporting on mortality were eligible for meta-analysis. Information was extracted from the reports of the retrieved studies, using a standardised data abstraction form. An odds ratio (OR) of postoperative infections or mortality in recipients of non-WBC-reduced RBC vs. WBC-reduced RBC or whole blood vs. WBC-reduced RBC or whole blood and a 95% confidence interval (CI) of the OR were calculated for each RCT.

An association with post-operative infection was demonstrated across RCT transfusing RBC, WBC-reduced after storage (summary OR = 2.25; 95% CI = 1.12-4.25) but not before storage (summary OR = 1.06; 95% CI = 0.91-1.24). An association of ABT with mortality was demonstrated across RCT conducted in cardiac surgery (summary OR = 1.72; 95% CI = 1.05-2.81) and across RCT transfusing buffy-coat-reduced RBC vs. RBC, WBC-reduced before storage (summary OR = 1.6; 95% CI = 1.14-2.24) but not across RCT transfusing non-buffy-coat-reduced RBC vs. RBC, WBC-reduced before storage (summary OR = 1.01; 95% CI = 0.73-1.40).

The meta-analysis presented in this article did not demonstrate an overall deleterious TRIM effect of ABT, although it did detect a TRIM effect across three specific subsets of RCT: studies transfusing to the control arm WBC-reduced RBC or whole blood filtered after storage and reporting on post-operative infections; studies conducted in cardiac surgery and reporting on short-term, all-cause mortality; and studies transfusing buffy-coat-reduced RBC to the treatment arm and WBC-reduced RBC, filtered before storage, to the control arm and reporting on short-term, all-cause mortality. Other associations found in subgroup analyses appear to contradict current theories about the pathogenesis of TRIM.

van Burik J-A, Brunstein CG
Infections complications following unrelated cord blood transplantation (review)

Umbilical cord blood (UCB) is an increasingly used source of haematopoietic stem cells (HSC) for transplantation of patients with advanced and high-risk haematological diseases. UCB has been shown to be an effective alternative for patients who lack a suitable adult donor, but has largely been limited by the ability of finding an UCB unit with an adequate cell dose, which has been shown to be an important determinant of outcomes. Newer strategies, however, such as double UCB grafts and non-myeloablative conditioning regimens, are expanding UCB transplantation (UCBT) to larger and older patient populations. The review by van Burik and Brunstein outlines infections present at death, the epidemiology of individual infections, and the impact of UCBT as HSC source. The Authors, from the University of Minneapolis (MN, USA), reviewed the most important studies published on the matter since 2000. Most studies found an approximate rate of infection at death of 30-40% among UCBT recipients. Bacterial infections are the most frequent source of serious infections after UCBT and the risk is higher for patients with graft failure. This kind of infection often occurs prior to the engraftment. In addition, there is a delayed recovery of the immune response among patients with graft-versus-host-disease (GvHD). Commonly identified bacteria include Gram-negative bacilli, such Pseudomonas, Acinetobacter, ...
and Escherichia coli, and Gram-positive organisms such as coagulase-negative Staphylococci, Enterococcus, and Staphylococcus aureus. As far as fungal infections are concerned, Aspergillus is the most frequent agent, accounting for about 50-70% of infections, and Candida species are the cause of most of the remaining fungal infections. Viral infections or reactivations (herpes viruses, cytomegalovirus, Epstein-Barr virus, respiratory viruses) tend to be observed starting after engraftment until immune reconstitution and their incidence is likely to be influenced by whether GvHD does or does not develop. Toxoplasmosis, as parasitic infection, has been reported following UCBT and appears to be associated with pre-transplantation seropositivity and high mortality.

The risk of serious infections among children receiving UCBT is, however, comparable to that of children receiving marrow HSC and is lower than that of recipients of T-cell-depleted stem cells. Among adults, despite an overall higher incidence of severe infections after UCBT that after unrelated donor grafts, non-relapse mortality and overall survival were not significantly different between recipients of HSC from the different sources.

The Authors conclude that further studies are needed to confirm these observations and correlate them with analyses of markers of immune reconstitution.


Severe acquired aplastic anaemia (SAA) is a potential fatal bone marrow failure syndrome, occurring mainly in children and young adults. Haematopoietic stem cell transplantation (HSCT) from unrelated donors is treatment of choice for patient affected with SAA, but this approach is limited by the availability of HLA-matched donors. Immunosuppressive therapy (a combination of anti-thymocyte globulin and cyclosporine A) is the alternative treatment for patients without a suitable related donor. HSCT from a phenotypically HLA-matched unrelated donor is indicated as salvage treatment for patients who fail to respond to one or more courses of immunosuppressive therapy and for patients who experience relapse of the disease. Historically, HSCT using unrelated donors for patients affected with SAA was associated with high mortality rates. The Authors of this report, on behalf of the French Society of Bone Marrow Transplantation and Cellular Therapy (SFCDM-TC), have analysed the outcome of 89 patients (median age 17 years, range 0-52) with acquired SAA, undergoing HSCT from an unrelated donor between 1989 and 2004, with the aim to verify whether, in recent years, the outcome of these transplantations improved, and, if so, to determine whether the improvement resulted from changes in selection of patients, changes in transplantation technique, or both. Reviewed cases were consecutively reported to the French Registry by 25 transplant centres.

The HLA matching between recipient and donor was based on HLA search determinants according to the standard techniques: serology and DNA typing. Two time periods were considered: 1989-1998 (37 transplants) and 1999-2004 (52 transplants). In the first period, most donors (91%) were matched at a generic level (or antigen level) for HLA-A, -B, -DR. In the second period, most of the pairs recipient-donor was matched at allelic level, with molecular analysis of HLA-A, -B, -C, -DRB1 and -DQB1 loci. Five-years survival probabilities (95% confidence interval) were different in the two groups: 29±7% and 50±7%, respectively (p<0.01). The main difference between the two cohorts concerned just HLA matching at allelic level, which was more frequent in 1999-2004 than in the former period (p=0.0004). In multivariate analysis, another factor affecting the survival was younger age of recipient (=17 years, p<0.0001). Overall survival reached 78±11% at five years for the younger, fully HLA matched recipients. Other factors were considered: SAA subtype (post-hepatic, idiopathic, paroxysmal nocturnal haemoglobinuria), therapeutic treatment, median age of donors, donor CMV serology, nucleated cell dose/kg, type of GvHD prophylaxis, but the differences between the two periods were not statistically significant.

In summary, from the French experience, survival after unrelated transplantation for SAA improved over the past 15 years. The reviewed data suggest that HLA matching at the allelic level was an important in this progress. Results for young patients who are fully HLA-matched with their donor are comparable to those observed after related HSCT.