2: Basics of blood groups and antibodies

Essentials

- ABO-incompatible red cell transfusion is often fatal and its prevention is the most important step in clinical transfusion practice.
- Alloantibodies produced by exposure to blood of a different group by transfusion or pregnancy can cause transfusion reactions, haemolytic disease of the fetus and newborn (HDFN) or problems in selecting blood for regularly transfused patients.
- To prevent sensitisation and the risk of HDFN, RhD negative or Kell (K) negative girls and women of child-bearing potential should not be transfused with RhD or K positive red cells except in an emergency.
- Use of automated analysers, linked to laboratory information systems, for blood grouping and antibody screening reduces human error and is essential for the issuing of blood by electronic selection or remote issue.
- When electronic issue is not appropriate and in procedures with a high probability of requiring transfusion a maximum surgical blood ordering schedule (MSBOS) should be agreed between the surgical team and transfusion laboratory.

There are more than 300 human blood groups but only a minority cause clinically significant transfusion reactions. The two most important in clinical practice are the ABO and Rh systems.

2.1: Blood group antigens

Blood group antigens are molecules present on the surface of red blood cells. Some, such as the ABO groups, are also present on platelets and other tissues of the body. The genes for most blood groups have now been identified and tests based on this technology are gradually entering clinical practice.

2.2: Blood group antibodies

These are usually produced when an individual is exposed to blood of a different group by transfusion or pregnancy (‘alloantibodies’). This is a particular problem in patients who require repeated transfusions, for conditions such as thalassaemia or sickle cell disease, and can cause difficulties in providing fully compatible blood if the patient is immunised to several different groups (see Chapter 8). Some antibodies react with red cells around the normal body temperature of 37°C (warm antibodies). Others are only active at lower temperatures (cold antibodies) and do not usually cause clinical problems although they may be picked up on laboratory testing.
2.3: Testing for red cell antigens and antibodies in the laboratory

The ABO blood group system was the first to be discovered because anti-A and anti-B are mainly of the IgM immunoglobulin class and cause visible agglutination of group A or B red cells in laboratory mixing tests. Antibodies to ABO antigens are naturally occurring and are found in everyone after the first 3 months of life. Many other blood group antibodies, such as those against the Rh antigens, are smaller IgG molecules and do not directly cause agglutination of red cells. These 'incomplete antibodies' can be detected by the antiglobulin test (Coombs' test) using antibodies to human IgG, IgM or complement components ('antiglobulin') raised in laboratory animals. The direct antiglobulin test (DAT) is used to detect antibodies present on circulating red cells, as in autoimmune haemolytic anaemia or after mismatch blood transfusion. Blood group antibodies in plasma are demonstrated by the indirect antiglobulin test (IAT). Nearly all clinically significant red cell antibodies can be detected by an IAT antibody screen carried out at 37°C (see section 2.7).

2.4: The ABO system

There are four main blood groups: A, B, AB and O. All normal individuals have antibodies to the A or B antigens that are not present on their own red cells (Table 2.1). The frequency of ABO groups varies in different ethnic populations and this must be taken into account when recruiting representative blood donor panels. For example, people of Asian origin have a higher frequency of group B than white Europeans. Individuals of blood group O are sometimes known as universal donors as their red cells have no A or B antigens. However, their plasma does contain anti-A and anti-B that, if present in high titre, has the potential to haemolyse the red cells of certain non-group O recipients (see below).

Table 2.1 Distribution of ABO blood groups and antibodies

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Antigens on red cells</th>
<th>Antibodies in plasma</th>
<th>UK blood donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>none</td>
<td>anti-A and anti-B</td>
<td>47%</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>anti-B</td>
<td>42%</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>anti-A</td>
<td>8%</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>none</td>
<td>3%</td>
</tr>
</tbody>
</table>

2.4.1: Transfusion reactions due to ABO incompatibility

ABO-incompatible red cell transfusion is often fatal and its prevention is the most important step in clinical transfusion practice (Chapter 5). Anti-A and/or anti-B in the recipient’s plasma binds to the transfused cells and activates the complement pathway, leading to destruction of the transfused red cells (intravascular haemolysis) and the release of inflammatory cytokines that can cause shock, renal failure and disseminated intravascular coagulation (DIC). The accidental transfusion of ABO-incompatible blood is now classified as a ‘never event’ by the UK Departments of Health.
Transfusion of ABO-incompatible plasma containing anti-A or anti-B, usually from a group O donor, can cause haemolysis of the recipient’s red cells, especially in neonates and small infants. Red cells stored in saline, adenine, glucose and mannitol (SAG-M) additive solution (see Chapter 3) contain less than 20 mL of residual plasma so the risk of haemolytic reactions is very low. Group O red cell components for intruterine transfusion, neonatal exchange transfusion or large-volume transfusion of infants are screened to exclude those with high-titre anti-A or anti-B. Group O plasma-rich blood components such as fresh frozen plasma (FFP) or platelet concentrates should not be given to patients of group A, B or AB if ABO-compatible components are readily available (Table 2.2). Cryoprecipitate contains very little immunoglobulin and has never been reported to cause significant haemolysis. In view of the importance of making AB plasma readily available, AB cryoprecipitate manufacture and availability is a low priority for the UK Blood Services.

Table 2.2 Choice of group of red cells, platelets, fresh frozen plasma (FFP) and cryoprecipitate according to recipient’s ABO group

<table>
<thead>
<tr>
<th>Patient’s ABO group</th>
<th>Red cells</th>
<th>Platelets</th>
<th>Fresh frozen plasma (FFP)</th>
<th>Cryoprecipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>First choice</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>Second choice</td>
<td>A</td>
<td>A or B</td>
<td>A or B</td>
</tr>
<tr>
<td></td>
<td>Third choice</td>
<td>AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>First choice</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Second choice</td>
<td>O&lt;sup&gt;c&lt;/sup&gt;</td>
<td>O&lt;sup&gt;d&lt;/sup&gt;</td>
<td>O or B</td>
</tr>
<tr>
<td></td>
<td>Third choice</td>
<td>B&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>First choice</td>
<td>B</td>
<td>A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Second choice</td>
<td>O&lt;sup&gt;c&lt;/sup&gt;</td>
<td>O&lt;sup&gt;d&lt;/sup&gt;</td>
<td>O or A</td>
</tr>
<tr>
<td></td>
<td>Third choice</td>
<td>A&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.5: The Rh system

There are five main Rh antigens on red cells for which individuals can be positive or negative: C/c, D and E/e. RhD is the most important in clinical practice. Around 85% of white Northern Europeans are RhD positive, rising to virtually 100% of people of Chinese origin. Antibodies to RhD (anti-D) are only present in RhD negative individuals who have been transfused with RhD positive red cells or in RhD negative women who have been pregnant with an RhD positive baby. IgG anti-D antibodies can cause acute or delayed haemolytic transfusion reactions when RhD positive red cells are transfused and may cause haemolytic disease of the fetus and newborn (HDFN – see Chapter 9). It is important to avoid exposing RhD negative girls and women of child-bearing potential to RhD positive red cell transfusions except in extreme emergencies when no other group is immediately available.

2.6: Other clinically important blood group systems

Alloantibodies to the Kidd (Jk) system are an important cause of delayed haemolytic transfusion reactions (see Chapter 5). Kell (anti-K) alloantibodies can cause HDFN and it is important to avoid transfusing K positive red cells to K negative girls and women of child-bearing potential. Before red cell transfusion, the plasma of recipients is screened for clinically important red cell alloantibodies so that compatible blood can be selected.

2.7: Compatibility procedures in the hospital transfusion laboratory

2.7.1: Group and screen
The patient’s pre-transfusion blood sample is tested to determine the ABO and RhD groups and the plasma is screened for the presence of red cell alloantibodies capable of causing transfusion reactions. Antibody screening is performed using a panel of red cells that contains examples of the clinically important blood groups most often seen in practice. Blood units of a compatible ABO and Rh group, negative for any blood group alloantibodies detected, can then be selected from the blood bank, taking into account any special requirements on the transfusion request such as irradiated or cytomegalovirus (CMV) negative components.

Almost all hospital laboratories carry out blood grouping and antibody screening using automated analysers with computer control of specimen identification and result allocation. This is much safer than traditional manual techniques and eliminates most transcription and interpretation errors. ABO grouping is the single most important test performed on pre-transfusion samples and the sensitivity and security of testing systems must never be compromised. Robust identification procedures outside the laboratory at patient blood sampling, collection of blood from the blood bank and administration of blood at the bedside are vital (see Chapter 4). The current British Committee for Standards in Haematology (BCSH) guideline for pre-transfusion compatibility procedures (2012) recommends that a second sample should be requested for confirmation of the ABO group of a first-time transfused patient provided this does not impede the delivery of urgent red cells or components (http://www.bcshguidelines.com).

2.7.2: Compatibility testing

Traditionally, the final step in providing safe blood is to carry out a serological crossmatch between the patient’s plasma and a sample of red cells from the units of blood selected for transfusion. This is performed by the IAT method at 37°C, looking for evidence of a reaction that would indicate incompatibility.

2.7.3: Electronic issue

This is sometimes known as computer crossmatching. Most hospitals now issue the majority of blood by this safe and rapid technique. It relies on the fact that if the patient’s ABO and RhD groups are reliably established, and a sensitive antibody screen is negative, the possibility of issuing incompatible blood is negligible. The laboratory computer can identify all compatible units in the blood bank inventory without the need for further testing. National guidelines require the use of automated testing systems interfaced with laboratory information systems before electronic selection is used and all results must be transmitted electronically to remove human error. Electronic issue should not be used:

- If the patient’s plasma contains, or has been known to contain, red cell alloantibodies of clinical significance
- If the antibody screen is positive
- If the patient has had an ABO-incompatible marrow or haemopoietic stem cell transplant
- If the patient has had an ABO-incompatible solid organ transplant in the last 3 months
- For neonates or fetuses, if the mother has an IgG red cell antibody present in her plasma.

2.7.4: Blood for planned procedures

Many operations rarely need transfusion. As long as the laboratory can provide components quickly in an emergency, there is no need to reserve blood units in the blood bank. Group and screen and electronic issue are now widely used in this situation and allow more efficient use of blood stocks and laboratory scientist time.

Patients undergoing planned procedures that may require transfusion, such as major surgery, ideally have samples for group and screen taken at preadmission clinics. Problems in providing compatible blood are then identified before admission to hospital. There is a (usually small) risk that the patient may develop new blood group alloantibodies between the time of initial testing and the date of operation, especially if they have recently been transfused or become pregnant. Having reviewed current evidence, the BCSH
guidelines for pre-transfusion compatibility procedures (Milkins et al., 2012) made the following pragmatic recommendations for timing of pre-transfusion blood samples:

- Testing should be performed on samples collected no more than 3 days in advance of the transfusion when the patient has been transfused or become pregnant within the preceding 3 months.
- An extension to 7 days may be considered for regularly/frequently transfused patients with no alloantibodies and pregnant women with no significant alloantibodies who need to have blood standing by for a potential obstetric emergency such as placenta praevia.

Remote issue of compatible blood components from satellite blood refrigerators electronically linked to the laboratory computer system allows safe and efficient provision of blood when the transfusion laboratory and operating theatres are on different hospital sites. Successful adoption of this approach requires close collaboration with the clinical team and clear local guidelines and policies.

When electronic issue is not available or appropriate and in procedures with a high probability of requiring transfusion a maximum surgical blood ordering schedule (MSBOS) should be agreed between the surgical team and transfusion laboratory. This specifies how many blood units will be routinely reserved (in the blood bank or satellite refrigerator) for standard procedures, based on audits of local practice. When developing an MSBOS it is usual to aim for a crossmatched to transfused ratio of no more than 3:1 and actual blood use should be audited and reviewed at regular intervals.