# A physician's guide to transfusion in autoimmune haemolytic anaemia

Patients with autoimmune haemolytic anaemia (AIHA) frequently have anaemia of sufficient severity as to require a blood transfusion. However, it is impossible to find compatible blood when, as is frequently the case, the autoantibody in the patient's serum reacts with all normal red blood cells. Further, the autoantibody may mask the presence of a red cell alloantibody capable of causing a haemolytic transfusion reaction. Optimal patient management in this clinical setting requires special compatibility test procedures in the transfusion service laboratory. Equally important is that clinicians must understand the principles of the compatibility tests performed. Provided appropriate compatibility tests are performed, the indications for transfusion in patients with AIHA are not significantly different than for similarly anaemic patients without AIHA. Communication between clinicians and laboratory personnel are important to review the urgency of transfusion and the compatibility test methods used to select the optimal unit of red blood cells for transfusion.

Transfusion of patients with autoimmune haemolytic anaemia (AIHA) presents a unique set of potential problems (Branch & Petz, 1999; Leger & Garratty, 1999; Garratty & Petz, 2002; Garratty *et al*, 2002; Petz & Garratty, 2004). When the patient has a broadly reactive autoantibody, as is generally the case, the transfusion service is likely to find that all units of red blood cells (RBCs) are incompatible, thus adding an element of uncertainty to the risk-benefit ratio of transfusion. To make appropriate clinical decisions in this setting, there should be good communication between clinicians and transfusion medicine personnel. Clinicians have a responsibility to understand the principles of compatibility test procedures in patients with AIHA and to understand their significance, and transfusion service personnel have an obligation to provide to the clinician information concerning the extent and effectiveness of the compatibility test procedures employed.

## Indications for transfusion in AIHA

Perhaps one of the most common mistakes in management of patients with AIHA is the reluctance to transfuse such patients because of uncertainty regarding the safety and effectiveness of RBC units that are 'incompatible' because of the presence of an RBC autoantibody. It is true that haemolytic transfusion reactions are expected to occur when incompatibility is due to clinically important alloantibodies (Jenner & Holland, 1996). However, experience indicates that when incompatibility is due only to the presence of a RBC autoantibody, the survival of transfused RBCs is generally about as good as that of the patient's own RBCs, and transfusion can be expected to cause significant temporary benefit (Salama *et al*, 1992; Garratty & Petz, 1993; Petz & Garratty, 2004). Thus, as long as appropriate compatibility procedures are performed to detect and identify RBC alloantibodies (Garratty & Petz, 1993, 2002; Branch & Petz, 1999; Leger & Garratty, 1999; Petz, 1999; Petz & Garratty, 2004) (see below), the indications for transfusion in patients with AIHA are not significantly different than for similarly anaemic patients without AIHA. The decision to transfuse does not depend on compatibility test results and, instead depends on an evaluation of the patient's need for transfusion (Salama *et al*, 1992; Garratty & Petz, 1993; Petz & Garratty, 2004).

Examples of patients denied transfusion in spite of clear indications have been reported by Conley *et al* (1980, 1982). These authors (Conley *et al*, 1982) described five patients with AIHA and reticulocytopenia who developed life-threatening anaemia but who were not transfused because their physicians were concerned that compatible blood could not be obtained. This was true although the patients' haematocrits were at a level of 8–10%! After transfer to a tertiary care medical centre, the patients were promptly transfused, a measure that the authors felt was unquestionably lifesaving.

## Principles of compatibility tests – a summary for clinicians

Much has been written about the detailed serologic approaches that are available to select the optimal unit of RBCs for patients whose AIHA makes necessary more complex compatibility test procedures than usual (Petz, 1996; Branch & Petz, 1999; Leger & Garratty, 1999; Garratty & Petz, 2002; Petz & Garratty, 2004). These must appear baffling to clinicians who have not had detailed training in the laboratory aspects of transfusion medicine. Understanding the principles of the specialized compatibility tests performed and their significance in minimizing the risk of transfusion will eliminate any undue reluctance by the clinician to transfuse. The following

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summary is intended to provide for clinicians an outline of the principles of compatibility testing for patients with AIHA, which can be used as a basis for communicating with transfusion service personnel.

## Compatibility tests in AIHA

Compatibility tests are performed to detect and identify antibodies that have the potential to cause a haemolytic transfusion reaction. Compatible RBCs do not react with red cell antibodies in the patient's serum. However, in many patients with AIHA, the autoantibody will react with all units of RBCs thereby making it impossible to provide compatible blood. Compounding the problem is the fact that the autoantibody may mask the presence of alloantibodies in compatibility tests.

The most important technical problem faced by the transfusion service regarding patients with AIHA relates to the detection of red cell alloantibodies in patients with a broadly reactive autoantibody. These alloantibodies are developed as a result of previous transfusions or pregnancies, and are capable of causing haemolytic transfusion reactions. They may be directed against antigens of a number of blood group systems, such as Rh, Kell, Kidd, and Duffy. Published data indicate that alloantibodies were detected in 209 of 647 sera (32%) of patients with AIHA (Branch & Petz, 1999), clearly indicating the need for a method to detect these antibodies to prevent alloantibody-induced haemolytic transfusion reactions. Indeed, undetected alloantibodies may be the cause of increased haemolysis following transfusion, which may be falsely attributed to an increase in the severity of AIHA (Petz, 1996; Petz & Garratty, 2004).

These alloantibodies are ordinarily detected and identified by testing the patient's serum against a panel of red cells of known phenotypes. For example, anti-Jk$^a$ (an antibody in the Kidd blood group system that can cause serious haemolytic transfusion reactions) is identified by the fact that the patient's serum will react with Jk(a+) RBCs and will not react with Jk(a−) RBCs. However, in warm antibody AIHA, the autoantibody in the patient's serum will generally react with all RBCs tested, thus masking the presence of the anti-Jk$^a$.

## Specialized procedures for detection of alloantibodies in patients with autoantibodies

A number of approaches are available for selecting donor RBC units for transfusion of patients who have warm autoantibodies (Petz, 1996; Branch & Petz, 1999; Leger & Garratty, 1999; Garratty & Petz, 2002; Petz & Garratty, 2004). (Compatibility testing in patients with cold antibody AIHAs is reviewed later.) The most effective of these are adsorption tests, which remove autoantibody from the patient's serum and allow for detection and identification of alloantibodies in the adsorbed serum. An alternative approach, which may be about as effective in avoiding the effects of alloantibodies, but which is not widely implemented in transfusion services, is to perform extensive RBC phenotyping of the patient and the donor units.

Other simple tests that provide a modicum of safety include routine testing of the patient's serum against a red cell panel and diluting the patient's serum before doing compatibility testing.

### *Testing the patient's serum or diluted serum against a red cell panel*

If a weakly reactive autoantibody and a strongly reactive alloantibody are present, the differences in the strength of the reaction of various cells of the panel will make this evident. In an attempt to dilute the serum so that the autoantibody will no longer react *in vitro*, one may select a dilution of the patient's serum that reacts 1+ against donor RBCs, and then test that dilution against a panel of RBCs (Leger & Garratty, 1999). However, there is no assurance that a patient's alloantibody will react more strongly than the autoantibody. These techniques are easy and rapid, but are unreliable (Leger & Garratty, 1999) so other, more effective procedures should be performed except in very urgent situations.

### *Adsorption procedures*

*Warm autoadsorption technique.* The optimal adsorption technique for detecting alloantibodies in the presence of a broadly reactive autoantibody is the warm autoadsorption procedure (Petz & Branch, 1983; Branch & Petz, 1999; Petz & Garratty, 2004). In this technique, some of the autoantibody is eluted from the patient's RBCs, as with 'ZZAP' reagent (Branch & Petz, 1982) (a mixture of 0·1 mol/l dithiothreitol plus 0·1% cysteine-activated papain or 0·1% ficin), and then these cells are used to adsorb the autoantibody from the patient's serum at 37°C. The adsorbed serum can then be tested for alloantibodies, since alloantibodies will not be adsorbed onto the patient's own RBCs.

A problem faced by transfusion services is that the patient's severe anaemia may preclude obtaining a large enough volume of RBCs for the autoadsorption procedure. Physicians should provide as many RBCs as may be reasonable because the autoadsorption procedure is the most effective method for detecting alloantibodies in patients with warm autoantibodies. The warm autoadsorption test is not useful in patients who have been transfused recently (within about the last 3 months) because even a small percentage of transfused cells may adsorb the alloantibody during the *in vitro* adsorption procedure, thus invalidating the results (Laine *et al*, 2000).

*Allogeneic adsorption.* When autoadsorption tests are not feasible because of an insufficient volume of the patient's RBCs or because of recent transfusion, the optimal procedure is allogeneic adsorption. In this procedure adsorption of autoantibody from the patient's serum is carried out using several samples of allogeneic red cells of varying phenotypes.

For example, performing an adsorption using a Jk(a−) cell, of a serum containing a warm autoantibody and an anti-$Jk^a$ alloantibody will remove the autoantibody but not the anti-$Jk^a$. By selecting two or three samples of RBCs of various phenotypes for the alloadsorption procedure, alloantibodies that are responsible for almost all clinically important haemolytic transfusion reactions can be detected (Branch & Petz, 1982; Petz & Branch, 1983; Petz & Garratty, 2004).

### *Transfusion of phenotypically matched RBC*

When extended phenotyping of the patient's RBCs is performed, it is possible to determine which alloantibodies a patient could develop as a result of previous transfusions or pregnancies. For example, if a patient is Jk(a+), it is impossible to develop an anti-$Jk^a$ alloantibody. Transfusion of RBCs that are selected on the basis of the patient's extended phenotype can provide a significant measure of safety (Shirey *et al*, 2002), but some caveats and precautions must be stressed (Garratty & Petz, 2002).

To provide adequate safety, typing must be performed for numerous RBC antigens (e.g. D, C, E, c, e, K, $Jk^a$, $Jk^b$, $Fy^a$, $Fy^b$, S, and s). However, determining the extended phenotype is technically difficult when the patient has a positive direct antiglobulin (Coombs') test and may be impossible in a significant percentage of patients with warm antibody AIHA even when attempted by the most skilled technologists. Partial phenotyping, e.g. for Rh, K, and $Jk^a$ antigens, would only provide protection against a limited number of alloantibodies that can cause haemolytic transfusion reactions (Garratty & Petz, 2002; Petz & Garratty, 2004), and therefore would not preclude the necessity of pretransfusion adsorption studies.

Whether implementation of this approach is cost-effective and feasible at many hospitals and blood centres has not been determined. If the intention is to emphasize providing phenotype-matched units, it must be determined that the blood supplier could readily provide such units, and it must be recognized that adsorption studies will be required in cases were the patient's RBCs cannot be phenotyped.

## Compatibility testing in cold antibody AIHAs

Compatibility testing in cold antibody AIHAs is less labour intensive than in warm antibody AIHA. In cold agglutinin syndrome, the autoantibody does not often react up to a temperature of 37°C, whereas clinically significant RBC alloantibodies will react at this temperature. Accordingly, the compatibility test can be performed strictly at 37°C (Petz & Garratty, 2004). If the transfusion service is not able to perform testing strictly at 37°C, one or two cold autoadsorptions should be done, which will not remove a high titer cold agglutinin completely, but are likely to eliminate reactions that occur at 37°C. Although the specificity of cold agglutinins is frequently anti-I, providing RBC negative for the I antigen is not practical because of their rarity, and their use may not be beneficial.

Similarly, in paroxysmal cold haemoglobinuria (PCH), the autoantibody will not react at 37°C. In this disorder, the autoantibody is unusual among AIHAs in that it very often has specificity for a RBC antigen, almost always the P antigen. Although the routine crossmatch test may appear to be compatible with P+ red cells since the antibody reacts only in the cold (usually <15°C), there are some suggestions that p or $P^k$ red cells (lacking the P antigen) will survive better (Rausen *et al*, 1975; Sabio *et al*, 1992). However, these RBCs are only available from rare donor files, and patients are likely to require transfusion before the RBCs can be obtained. Generally, transfusion of RBCs of common P types should be provided since patients with PCH often have severe haemolysis, and waiting for p or $P^k$ RBCs is likely to delay a needed transfusion. Successful transfusion of patients with PCH has been reported by numerous authors and, almost certainly, the transfusions were of P-positive blood (Dacie, 1992).

## 'Least incompatible' units

The term 'least incompatible' unit seems to be used very frequently in transfusion services (at least in the USA), although it is not defined in the medical literature and is used differently by different transfusion medicine professionals (Garratty *et al*, 2002; Petz, 2003; Petz & Garratty, 2004). It is probably true that the term lingers on from decades ago before techniques for detecting alloantibodies in the presence of autoantibodies had been introduced. Adsorption procedures, as reviewed above, were not described until the 1960s (Dorner *et al*, 1968) and were not widely implemented until later (Petz & Garratty, 1975, 1980; Morel *et al*, 1978). Prior to this time, when a patient with AIHA had a serum autoantibody that reacted with all cells in the crossmatch test, the transfusion service would merely select a number of ABO compatible units, test the reactivity of the patient's autoantibody against them, and select the unit that reacted least strongly (Yunis & Bridges, 1966).

Such a procedure for selecting 'least incompatible' units must not be considered an acceptable alternative to the techniques described above for selecting donor units for transfusion of patients with AIHA. This process as the sole means of selecting RBCs for transfusion of such patients will not reliably detect alloantibodies and is unacceptable in modern day transfusion medicine. This is a dangerous practice and should be abandoned, except in extremely urgent settings in which there is not time to perform adequate serologic tests.

It is true that there is no evident disadvantage to selecting the unit that reacts least strongly from among those selected for transfusion on the basis of adsorption tests or extended RBC phenotyping. This may seem to provide some additional assurance that an alloantibody has not been missed, although this is unlikely after an appropriate search for alloantibodies, as described above, has been carried out. One may also argue that the patient's autoantibody may react more strongly with some unidentified RBC antigen(s) than others. However, some

variability in reactivity caused by an autoantibody can be expected to occur when a number of units are crossmatched simply because of the limitations of precision of serologic reactions. If no specificity of the autoantibody can be determined, modest differences in the variability in reactivity are not likely to be of significance. Indeed, even if some specificity of the autoantibody can be determined, data indicate this is not always a significant factor in RBC survival after transfusion in AIHA (Petz & Garratty, 2004).

Therefore, although it is conceivable that some benefit may ensue by selecting a 'least incompatible' unit from among those selected for transfusion on the basis of adsorption tests, this is not likely. Further, the use of the term in discussion with clinicians can lead only to confusion and a lack of confidence in the safety of units selected by the transfusion service for transfusion to a patient with AIHA. This, in turn, may lead to avoiding transfusion in a situation where transfusion is needed. The use of the term 'least incompatible' unit should be discarded (Garratty *et al*, 2002; Petz, 2003; Petz & Garratty, 2004).

## When the transfusion is urgent

Adequate testing for alloantibodies in a patient with AIHA may take 4–6 h, or even longer if testing must be performed at a referral laboratory. The clinician must balance the risk of withholding transfusion for that length of time with the benefit of added safety that complete testing provides against alloantibody-induced haemolytic transfusion reactions. In this situation, the following considerations are of significance.

One should keep in mind that the probability that alloantibodies will be present in a person who has not previously been transfused or pregnant is very low. In fact, only a few percent of all hospitalized patients have RBC alloantibodies so that if transfusion is extremely urgent, the lesser risk may be to transfuse rather than waiting for completion of the compatibility testing. Even in very urgent situations, however, there is almost always time to perform at least some of the recommended compatibility test procedures for patients with AIHA. The quickest, but least reliable, techniques for detection of alloantibodies are the dilution technique and partial RBC phenotyping.

If somewhat more time is available, the warm autoadsorption test should be performed since it is highly effective for detection and identification of alloantibodies and only requires only one to three adsorptions of the patient's serum with the patient's (ZZAP-treated) RBCs.

The most time-consuming procedure, which may take about 4 h, is allogeneic adsorption, which is indicated if the patient has been transfused recently or if the patient's RBCs are not available for autoadsorption. In order to expedite such testing, transfusion services should plan ahead and have available several millilitres of packed RBCs of each type that is needed. For example, one may use three samples of allogeneic RBCs, one rr, one $R_1$ $R_1$ and one $R_2$ $Rc_2$; one sample should be Jk(a−) and one should be Jk(b−). The RBCs can be stored in the frozen state or, if their need is reasonably frequent, may be stored in anticoagulant for use for up to 6 months (Petz & Garratty, 2004).

## Communication between the clinician and the transfusion service

### *Responsibilities of the clinician*

A discussion between the attending physician and the transfusion service should take place as soon as it is evident that a patient with AIHA is being considered for transfusion (Jefferies, 1994; Petz, 2003; Petz & Garratty, 2004). The clinician should indicate the urgency of the transfusion and discuss with the transfusion service personnel the time required for the more detailed than usual serologic studies that will be necessary. The clinician should also discuss the compatibility tests to be undertaken by the laboratory using the above outline of compatibility test procedures as a guide to adequate pretransfusion testing, and seek assurance that appropriate testing is to be performed.

### *Responsibilities of the transfusion service*

In some instances, it will be the responsibility of the transfusion service to initiate the communication since the diagnosis of AIHA may first be made during compatibility testing for a requested transfusion. In any case, the transfusion service should feel obligated to supply the clinician with information about the compatibility test procedures performed. After appropriate testing, the clinician should be assured that transfused RBCs are unlikely to cause an acute haemolytic transfusion reaction even though the RBCs cannot be expected to survive normally because of the patient's autoantibody. The attending physician can then proceed to make a decision regarding transfusion on the basis of the clinical need.

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