Solving cases in autoimmune haemolytic anaemia

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Autoimmune haemolytic anaemia (AIHA) can be classified in different types including warm AIHA (WAIHA), cold haemagglutinin disease (CHAD), mixed-type AIHA, paroxysmal cold haemoglobinuria and drug-induced AIHA. Warm AIHA (WAIHA) is the most common type of AHAI characterized by warm autoantibodies primarily IgG (rarely IgM or IgA) reacting best at 37°C. Most are directed against the Rh protein, and over 90% of cases have a positive Direct Antiglobulin Test (DAT). The patient's plasma contains autoantibodies that appear as a panagglutinin, and the biggest concern is that they mask the presence of alloantibodies. It is important to rule out, or identify, alloantibodies in the plasma/serum, and identification of the autoantibody specificity is rarely useful. The serologic work required to detect and identify underlying alloantibodies may include multiple autologous adsorptions and elutions, and once a patient is transfused, alloantibody identification becomes more difficult, requiring allogeneic adsorptions. Molecular testing is also being used as a valuable method to predict the extended red cell antigen profile in order to select antigen-negative red-blood-cells for adsorption of autoantibodies when searching for underlying alloantibodies and to provide extended matched units to the patients. Considering that patients with AIHA become alloimmunized much more commonly than other patients, it is very important that efficient procedures for detecting underlying alloantibodies, although labour-intensive, must be used in pretransfusion testing.

Key words: antigens and antibodies, blood groups, genotyping, immunoglobulins, immunohaematology, red blood cell

Introduction

Autoimmune haemolytic anaemia (AIHA) is caused by the production of 'warm-' or 'cold-' reactive antibodies directed against antigens on the patient’s red blood cells (RBCs). Autoantibody production can be triggered by disease, viral infection or drugs; from breakdown in immune system tolerance to self-antigens; or from exposure to foreign antigens that induce antibodies that cross-react with self-RBC antigens [1–3]. Autoantibody specificity is not always obvious because patient’s antigen expression can be depressed when autoantibody is present. AIHA can be classified in different types including warm AIHA (WAIHA), cold haemagglutinin disease (CHAD), mixed-type AIHA, paroxysmal cold haemoglobinuria (PCH) and drug-induced IHA (Table 1). AIHA cases can be a challenge to transfusion services and immunohaematology reference laboratories, and a systematic approach is required to solve difficult cases of warm, cold and drug-induced autoantibodies and to optimize the process in order to provide appropriate transfusion support for patients with AIHA.

Warm autoimmune haemolytic anaemia

Warm AIHA is the most common type of AHAI characterized by warm autoantibodies primarily IgG (rarely IgM or IgA) reacting best at 37°C. Most are directed against the Rh protein, but autoantibodies to Wr, Kp, Jk and U antigens have been reported [2]. Of WAIHAs, about one-third are idiopathic. The other two-thirds are secondary to conditions such as systemic lupus erythematosus (SLE), other autoimmune diseases and lymphoproliferative disorders [2–5].

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Solving cases of WAIHA

Over 90% of cases have a positive DAT. The antibody screen is positive in up to two-thirds of cases (and more than 80% with proteolytic enzymes), and in up to 20% of cases, there is a concurrent cold autoantibody [2–5].

Red cell phenotyping for all common antigens except Fya and FYb can be carried out at room temperature in WAIHA using IgM monoclonal reagents. For FY antigens and high-incidence antigens, a pretreatment of RBCs with chloroquine diphosphate or EDTA glycine acid is used to remove the autoantibodies [6]. Although haemagglutination remains the gold standard in immunohaematology reference laboratory, DNA methods are valuable to predict the type of a patient with WAIHA when direct agglutinating antibodies are not available, IgG removal by chemical treatment of RBCs is insufficient and the patient was recently transfused [7].

The autoantibody specificity in the eluate is clearly defined in less than 10% of cases; the remainder have panagglutinins possibly with relative Rh specificity (20%), broad Rh specificity (>70%), broad KEL specificity or other (occasionally). The patient’s plasma contains autoantibodies, and the biggest concern is that they mask the presence of alloantibodies [6, 8, 9]. Evidence of immune haemolysis, transfusion history and pregnancy, diagnosis and medications are important information to direct the decision-making pathway for determining what further studies are necessary and how quickly they must be performed. The serologic work required to detect and identify underlying alloantibodies may include dilution, multiple autologous adsorptions, elutions, and once a patient is transfused, alloantibody identification becomes more difficult, requiring allogeneic adsorptions. Dilution of the autoantibody (typically 1:5) is proposed for laboratories that do not have the capability to perform adsorptions; however, only alloantibodies whose titre is higher than the autoantibodies’ titre will be detected. When the patient has a sufficient volume of RBCs and has not been recently transfused, autologous adsorption is the best technique to remove autoantibodies for detection of underlying alloantibody. Several methods (ZZAP, chloroquine diphosphate, EDTA glycine acid) are available to free the bound IgG from the red cells, making antigen sites available for in vitro antibody adsorption. Enzyme-treated cells and untreated RBCs in the presence of PEG can also be used to enhance the capacity of the RBCs to adsorb antibody [10]. Detailed procedures and limitations for using these methods for adsorptions and for pretreatment of red cells to determine antigen typing are available [9, 11, 12]. If the patient is severely anaemic, has recently been transfused, or if autoadsorption is unsuccessful, allogeneic adsorption procedures should be considered. If a patient’s phenotype is known or can be determined serologically, phenotypically matched allogeneic cells can be used to perform the adsorption but when the phenotype is undetermined, genotype-matched allogeneic cells should be used. For patient samples with very high titres of autoantibodies, subsequent adsorptions may be required. Once the plasma no longer reacts when tested with autologous red-blood-cells, an antibody screen and/or antibody identification panel can be performed to detect and identify any underlying alloantibodies. Although infrequent, a limitation of allogeneic adsorptions is that antibody to high-incidence antigens will be adsorbed out with almost all cells.

Considering that patients with AIAH become alloimmunized much more commonly than other patients, it is very important that efficient procedures for detecting underlying alloantibodies, although labour-intensive, must be used in pretransfusion testing [13]. In some cases of WAIHA, the target antigen may be weakened to the extent that the patient’s RBCs are negative in the DAT. The following antigens have been implicated: AnWj, Co3, En3, Ge3, JMH, Jka, Jkα, Kpα, LW, Rh, Sc1, Sc3, U and Vel [6].

Figure 1 depicts an investigation of WAIHA and Fig 2 depicts an antibody identification with positive DAT.

Additional techniques are available to investigate cases of immune-mediated haemolysis where DAT appears negative by routine methods [14].

Selecting blood for patients with WAIHA

It is important to rule out, or identify, alloantibodies in the plasma/serum (autoadsorption or differential adsorption is considered best and dilution, although easy is risky). Identification of the autoantibody specificity is rarely useful.

RBC components should be ABO and RhD compatible and lack antigens corresponding to any clinically relevant alloantibody(ies). Transfusing the least incompatible blood is rarely of value and one common strategy is to provide extended phenotype-matched blood, but this approach is only applicable when the patient does not have a strongly positive DAT or has not been transfused [15]. Matching the
genotype of donor and patient can also replace allo- and autoadsorptions by providing the most highly matched blood to the patients with AIHA without heavy, costly, lengthy and sample consuming adsorption procedures [7]. RBC genotyping makes the selection of compatible blood easier, even in an emergency context. We have shown that after DNA typing, the patients who received antigen-matched RBCs by genotype had better in vivo survival, as assessed by rises in haemoglobin levels and diminished frequency of transfusions [16]. However, adsorption methods may still be needed when the patient’s genotype does not allow antigen-matched units selection.

Cold haemagglutinin disease

Of CHAD cases, about 10% have idiopathic chronic cold agglutinin disease due to IgM monoclonal antibodies. The patients are usually older than 50 years. The other 90% are secondary and can be (1) acute induced by post-infection (mycoplasm, CMV, EB) and are due to IgM polyclonal antibodies, or (2) chronic and associated with lymphoproliferative diseases (usually elderly patients) and are due to IgM monoclonal antibodies. Post-infection is the most common, with an antibody titre of less than 500 at lower temperatures. There may be extravascular haemolysis. The lymphoproliferative disease association is less common with an antibody titre of usually greater than 500 over a wider amplitude. Sometimes intravascular haemolysis is evident [17].

Solving cases of CHAD

Cold-reactive autoantibodies are primarily IgM. They react best at temperatures below 25°C but can agglutinate
RBCs or activate complement at or near 37°C, causing haemolysis or vascular occlusion upon exposure to cold. Patients with CHAD often have C3d on their RBCs, which can provide some protection from haemolysis. Most cold-reactive autoantibodies have anti-I specificity. Anti-I, anti-H, anti-Pr, anti-P or other specificities are much less common [2]. The serological findings include a positive DAT in more than 90% of cases, due to C3d coating the RBCs. An elution is unnecessary since complement cannot be eluted. The antibody screen is positive for cold panaglutinin in virtually 100% of cases and the specificity is usually anti-I or anti-IH, but some are anti-Pr, anti-P or anti-I. When present, cold autoantibodies, as with warm autoantibodies, can mask the presence of alloantibodies [2]. To detect alloantibodies in the plasma, it is often necessary to autoadsorb the patient’s plasma/serum. The parameters for cold autoadsorptions are basically the same as those detailed above for warm autoadsorptions. The serological features are summarized in Table 2, and a comparison of the characteristics of pathologic (CHAD) and harmless types of cold autoantibodies is given in Table 3. Figure 3 depicts an investigation of CHAD.

Selecting blood for patients with CHAD

Prior to transfusion, the ABO and RhD type are determined. This is facilitated by washing RBCs with warm saline to disperse the autoagglutination. On rare occasions, the autoagglutination is so strong that the RBCs need to be treated with low concentrations of a thiol compound (e.g. DTT, 2-ME). To rule out or identify alloantibodies in patient’s plasma/serum, it may be necessary to perform cold autoadsorption or alloadsorption. Identification of the autoantibody is rarely useful, and the titre of the agglutinin is more of interest than a clinical necessity.

If transfusion is deemed necessary, ABO and RhD compatible components lacking antigens corresponding to any alloantibody specificity present in the plasma/serum should be used. A cross-match performed in the warm may be helpful but can lead to false-negative results. Transfuse components through a blood warmer.

**Mixed-type autoimmune haemolytic anaemia**

This is a condition that can be hard to define. The patient’s DAT is positive with both IgG and C3d coating the RBCs. The plasma contains both warm-reactive IgG and cold-reactive IgM antibodies. Unlike CHAD, the cold agglutinin titres are usually less than 64, but the thermal amplitude is high. The patient often presents with severe haemolysis. It appears that the simultaneous presence of warm autoantibodies and cold autoantibodies, even though not particularly strongly reactive, causes severe anaemia [17, 18]. The patients frequently respond to corticosteroid therapy. This type of IHA is not uncommon in patients with SLE (greater than 25% of patients and as high as 42% have been reported) [4]. The serological features are summarized in Table 2.
Paroxysmal cold haemoglobinuria

The biphasic cold-reactive IgG antibody associated with PCH, the so-called Donath–Landsteiner antibody typically reacts with the high-prevalence antigen P (GLOB). It attaches to RBCs in the cold and very efficiently activates complement before it dissociates at warmer temperatures. This condition is most frequently seen in children as a consequence of a viral infection while the idiopathic type is rare and occasionally it occurs with syphilis [19, 20].

Solving cases of PCH

The DAT is weakly positive in more than half of the cases, usually due to IgG but sometimes to C3d. The eluate is often non-reactive. The antibody screen is weakly positive due to an antibody that appears as a panagglutinin but has anti-P or anti-IH specificity. The Donath–Landsteiner test (the antibody binds to RBCs at 0°C, which in the presence of complement haemolysis when warmed to 37°C) will be positive and is a characteristic of the condition [9, 11]. The serological features are summarized in Table 2.

Drug-induced immune haemolytic anaemia

When a positive DAT result is being evaluated and all previously discussed aetiologies have been eliminated, prescription medication should be assessed as a possible reason for haemolysis. The great majority result from the second and third-generation cephalosporins, cefotetan and ceftriaxone [21]. The main mechanisms of pathogenesis of drug-induced AIHA are adsorption of the drug onto RBCs (haptenic mechanism), adsorption of immune complexes onto RBCs (drug dependent), RBC membrane modification (drug-induced) and AIHA (drug-induced autoimmunity) [22, 23]. The serological features of the different types are summarized in Table 2.
Solving cases of drug-induced AIHA

The drug adsorption type is characterized by extravascular haemolysis and a positive DAT due to IgG. The eluate is non-reactive with panel RBCs and reactive with RBCs pretreated with the causal drug (e.g. high-dose penicillin and related drugs) [2, 24]. Haemolysis stops when the drug withdrawn.

In the drug-dependent type, haemolysis may be intravascular; and the DAT is positive usually due to C3d and rarely IgG sensitization. The plasma/serum is reactive with RBCs in presence of the causal drug (cephalosporin drugs are common culprits, but there is a long list of drugs that have been implicated) [25]. Haemolysis stops when the drug is withdrawn.

In the membrane modification type, the DAT is weakly positive due to ‘non-specific’ IgG and C3d binding. The antibody screen and eluate are non-reactive. Cephalothin and cisplatin are common causal drugs [2, 24, 25].

The drug-induced AIHA type is characterized by extravascular haemolysis, and a positive DAT due to IgG and often also with some C3d coating. The eluate is reactive with panel RBCs often appearing as a panagglutinin as in WAIHA – even sometimes with similar relative specificities, such as anti-e. Haemolysis persists after withdrawal of the drug. Methylldopa (now rarely used), levodopa and procainamide are causal drugs [2, 24, 25].

Numerous drugs have been described to cause drug-induced AIHA [25, 26], and the immunohaematologist is
well advised to be aware of them when investigating a case of haemolysis. Serologic methods for testing for drug antibodies are available [11, 24].

Conclusions

When developing a systematic approach to solve difficult cases of AIHA, considerations for serological and molecular testing, clinical indications and symptoms, laboratory results, transfusion and drug histories, knowledge in immunohaematology and common sense should direct the case resolution. Despite advances in transfusion medicine, DAT and serum studies correlated with the patient’s medical history are still the foundation for a decision matrix of further testing. Given the known limitations of serologic testing in patients with WAIHA, molecular testing is being used for the determination of minor red-cell blood group antigens in order to select antigen-negative RBCs for alloimmunizations and to provide extended matched units to the patients. Provision of extended matched blood in these patients can avoid transfusion of blood incompatible for clinically significant antibodies that could be present in the patient’s plasma. This strategy can also prevent alloimmunization, simplify future work ups and improve the safety of future transfusions.

Conflict of interests

The author declares no conflict of interests.

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