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TRANSFUSION SUPPORT AND PRE-TRANSFUSION TESTING IN AUTOIMMUNE HAEMOLYTIC ANAEMIA

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ABSTRACT

Autoimmune haemolytic anaemia (AIHA) is characterized by an increased destruction of red blood cells due to immune dysfunction and auto-antibody production. Clinical manifestations are mainly related to anaemia, which can become life-threatening in case of acute haemolysis. Aiming at counterbalancing severe anaemia, supportive treatments for these patients frequently include transfusions. Unfortunately, free serum auto-antibodies greatly interfere in pre-transfusion testing, and the identification of compatible red blood cell units for AIHA patients can be challenging or even impossible. Problems faced in pre-transfusion testing often lead to delay or abandonment of transfusions for AIHA patients. In this review, we discuss publications concerning global transfusion management in AIHA, with a focus on pre-transfusion testing, and practical clues to manage the selection of transfusion units for these patients. Depending on the degree of transfusion emergency, we propose an algorithm for the selection and laboratory testing of units to be transfused to AIHA patients.

Highlights

- Recent publications and recommendations suggest that, in adult patients presenting severe autoimmune haemolytic anaemia, transfusion support should not be delayed in lifethreatening clinical situations.
- Depending on the degree of emergency, only minimum or more specific laboratory testing should be performed to avoid delay in transfusion support.
- Recommendations for transfusion management in the laboratory and blood bank are summarized in the proposed algorithm.

INTRODUCTION TO AUTOIMMUNE HAEMOLYTIC ANAEMIA

Autoimmune haemolytic anaemia (AIHA) designates different types of acquired pathologies that are characterized by an increased destruction of red blood cells (RBCs), caused by auto-antibodies directed

against RBC membrane antigens. Primary and secondary AIHA forms can be distinguished, based on the absence or presence of another pre-existing condition that causes immune dysfunction [1, 2]. From a serological point of view, warm-type, cold-type and mixed-type AIHA can be distinguished.

Warm-type AIHA is the most frequent form, representing 60%–70% of AIHAs and generally involving polyclonal IgG-class antibodies that present maximum antigen binding at 37°C and frequently recognize antigens of the RH system. As the complement system is only moderately activated, RBC destruction mainly occurs through extravascular haemolysis. Clinical manifestations are mainly related to anaemia, with a possible silent disease onset as long as anaemia is counterbalanced by physiological compensatory mechanisms. In case of severe anaemia, symptoms can go up to heart failure [3, 4].

Among cold-type AIHAs (20%–25% of AIHAs), the most frequent presentations are cold agglutinin disease (CAD) and cold agglutinin syndrome (CAS). These clinical conditions involve mono- or oligoclonal IgM-class antibodies that optimally bind to RBC antigens in vitro at 3–4°C. Intravascular haemolysis occurs as IgM molecules directly fix and activate the complement system. To a lesser extent, extravascular haemolysis occurs through opsonization. Haemolysis/anaemia can be chronic if auto-antibodies present high thermal amplitudes (20–37°C) or occur as acute haemolytic episodes after cold exposure if auto-antibodies present low thermal amplitudes (<20°C).

CAD is defined as a primary cold-type AIHA, but is associated in almost all cases with a silent B-cell lymphoproliferative disorder which causes monoclonal IgM production. CAS designates secondary forms that present similar characteristics but occur as a complication of another disease (infections, malignancies, etc.) or vaccination. Especially, infection-related CAS can occur in young people/children, leading to a transient haemolytic episode and, in the vast majority of cases, a spontaneous resolution [5–8].

Paroxysmal cold haemoglobinuria (PCH) is a rare cold-type AIHA involving an auto-antibody called Donath–Landsteiner biphasic haemolysin [9]. This cold-reactive IgG-type antibody presents a high complement-activating potential, leading to intravascular haemolysis. Most PCH cases are infection-related secondary forms [10, 11].

Mixed-type AIHA (5%–10% of AIHAs) is characterized by the simultaneous presence of warm-reactive and cold-reactive autoantibodies. Cold-reactive antibodies in mixed type generally display higher thermal amplitudes (≥30°C) and higher antibody titres compared to CAD/CAS. Some cases present very severe and fulminating haemolytic anaemia [12–14].

Particular management is required in forms secondary to allogeneic haematopoietic stem cell transplantation (HSCT), which has a relatively high incidence of secondary AIHA, frequently presenting severe and treatment-refractory profiles [15, 16].

In this article, we review the transfusion support in AIHA and challenges faced in laboratories that perform pre-transfusion testing. We will discuss answers as to ‘when’ and ‘how’ to transfuse AIHA patients, corroborated with our own experience in the transfusion laboratory of Liège University Hospital in Belgium.

DIAGNOSTIC APPROACH AND LABORATORY TESTS IN AIHA

When a patient presents with anaemia in the absence of recent/active bleeding, investigations need to identify whether the cause is related to a production deficit or to an increased RBC destruction (i.e., haemolysis), which can be of non-immune or immune origin.

If immune-mediated RBC destruction is suspected, biological assessment first needs to confirm that haemolysis is occurring [17, 18]. Antibodies bound to the RBC surface can be detected by a direct antiglobulin test (DAT), which allows the in vitro identification of RBC sensitization occurring in vivo. RBC membranes can be sensitized by nonagglutinating antibodies (mostly IgG) that bind to RBC surface antigens and/or by molecules resulting from complement activation (C3c/C3d fractions). In a positive test, polyspecific (anti-IgG/C3) or monospecific anti-human globulin leads to RBC agglutination [19, 20]. Binding of IgM molecules to RBC surface is not very stable; thus in the vast majority of cases, a C3-positive DAT implies prior IgM binding and complement activation, while the monospecific IgM DAT remains negative.

AIHA is suspected when immune-mediated haemolysis is confirmed while the presence of allogeneic antibodies and RBCs (i.e., stem cell transplantation, transfusion, organ transplantation or foeto-maternal incompatibility) can be excluded. The most common DAT results in different AIHA presentations are summarized in Table 1 [1, 17].

Some AIHA patients (5%–10%) display a negative DAT result. Possible explanations include low-titre auto-antibodies, low-affinity IgG that detach in vitro from RBCs or atypical AIHA involving immunoglobulin types that are not detected by first-line anti-IgG/C3 reagents [21, 22]. Conversely, other patients can present a positive DAT and no haemolysis. COVID-19 patients present a higher prevalence of positive DAT results, associated with more severe forms of infection and anaemia [23, 24], but the association of autoimmune haemolysis in these patients remains more controversial [23–29].

Indirect antiglobulin test (IAT) reveals the presence of free antibodies after incubating a patient's serum with test RBCs of known phenotypes, followed by the addition of anti-human globulin. After a first-line IAT screening, larger panels are required to identify antibody specificity. In AIHA patients, free serum auto-antibodies can strongly interfere in these tests.

An elution of the RBC-sensitizing antibody can be performed in order to identify its specificity or panreactive profile. In AIHA patients presenting a 'negative' DAT result, elution might confirm the presence of an auto-antibody and is thus recommended in front of an unexplained haemolysis [2].

TABLE 1. *Types of autoimmune haemolytic anaemia and most frequent direct antiglobulin test results.*

AIHA type	Antibody type	DAT result	Eluate reactivity
Warm-type AIHA	IgG or IgA (rare)	IgG ± C3 or IgA (rare)	Panreactive
Cold-type AIHA	IgM	C3	Non-reactive
Mixed-type AIHA	IgG + IgM	IgG + C3	Panreactive
PCH	IgG (cold-reactive)	C3	Non-reactive

Abbreviations: AIHA, autoimmune haemolytic anaemia; DAT, direct antiglobulin test; PCH, paroxysmal cold haemoglobinuria.

DAT, IAT and elution tests that allow AIHA diagnosis are illustrated in Figure 1.

If DAT result is C3-positive, cold-type AIHA diagnosis is confirmed if the cold agglutinin titre at 4C is greater than or equal to 1/64, below which cold agglutinins have usually no clinical relevance. This threshold represents a compromise for eliminating the majority of patients who present cold agglutinins without clinical relevance. In addition, thermal amplitude and antigen specificity should be determined. In most cases, clinically significant antibodies present higher thermal amplitudes (25–30°C) than non-relevant cold antibodies [2, 17, 30], and the vast majority of primary AIHA and secondary forms related to Mycoplasma pneumoniae infection present anti-I specificity [31].

PCH diagnosis can be complex and needs to be confirmed by a Donath–Landsteiner test through sequential incubations at different temperatures [10, 32].

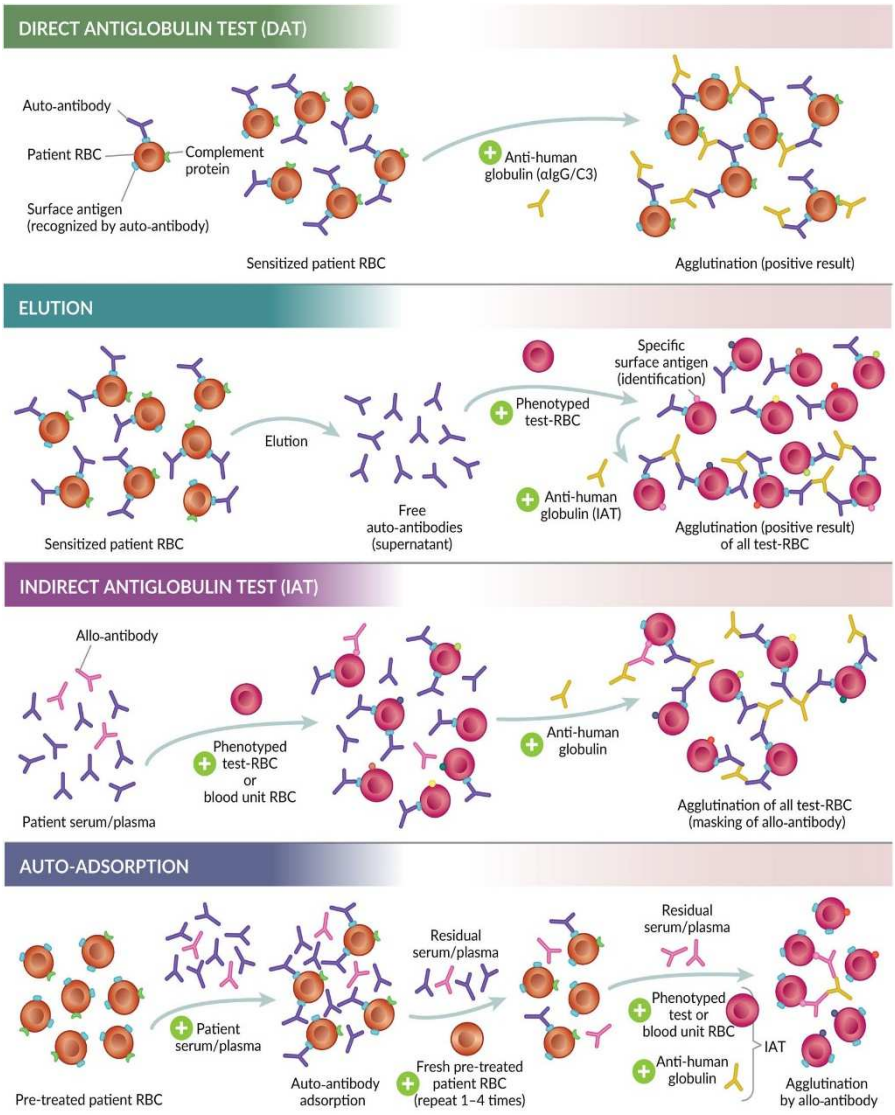
If AIHA diagnosis is confirmed, international consensus recommendations [17] suggest further investigations in order to exclude the presence of certain diseases that cause secondary AIHA. For these forms, it is essential to treat rapidly the underlying disease in order to obtain AIHA regression [15].

TRANSFUSION SUPPORT AND PRETRANSFUSION TESTING IN AIHA

RBC transfusions are often required as supportive therapy in order to counterbalance severe anaemia and avoid hypoxia in all forms of AIHA, possibly in combination with other supportive therapies such as plasma exchange, erythropoiesis-stimulating agents and/or intravenous immunoglobulin (IVIg) administration [17]. In pre-transfusion testing, the final step is an in vitro compatibility test. A negative result indicates the absence of recipient reactivity against the RBCs (= compatible units), whereas a positive result indicates reactivity towards the tested RBC unit, qualified as ‘incompatible’ for this patient. When an allo-antibody has been identified, the units selected for transfusion must be negative for the corresponding antigen.

Unfortunately, in a patient presenting with AIHA it can be extremely difficult or even impossible to obtain compatible RBC units because of the various interferences in pre-transfusion tests.

***FIGURE 1**. Laboratory tests performed in autoimmune haemolytic anaemia (AIHA). Direct antiglobulin test (DAT) reveals the in vivo sensitization of patient's red blood cells (RBCs) by the auto-antibody and/or complement proteins (C3c/d). In the figure, a polyspecific anti-IgG/ C3 anti-human globulin is represented. Elution of antibodies from sensitized patient RBC allows the identification of a panreactive auto-antibody, which causes agglutination of all test RBC regardless of their specific phenotypes. Indirect antiglobulin test (IAT) reveals the presence of free antibodies present in patient serum/plasma through the agglutination of specific phenotyped test RBC or RBC from specific blood units. In AIHA, all test RBCs are agglutinated by the panreactive auto-antibody, with the risk of masking the presence of a specific allo-antibody. Auto-adsorption technique reduces interferences by adsorbing auto-antibodies on autologous, pre-treated patient RBCs throughout several adsorption cycles (2–5). Residual serum/plasma can be used for IAT on phenotyped test RBC and blood unit RBC, allowing the identification of an underlying alloantibody.*



PROBLEMS WITH PRE-TRANSFUSION TESTING AND RECOMMENDATIONS

Auto-antibodies from AIHA patients also react with allogeneic RBCs if these display the corresponding antigens, usually high-frequency or 'public' antigens present in at least 99% of the random population. Thus, both the tests involving phenotyped test RBCs and RBCs to be transfused may present interferences or even all positive results, with the major risk of masking the presence of clinically significant alloantibodies. Among patients with auto-antibodies, approximately 30%–50% have been reported to have an underlying allo-antibody. In subjects with no history of pregnancy or transfusion, this risk is very low [2, 33–35].

Thus, transfusions for AIHA patients often present an analytical challenge. Specific attempts to reduce interferences can take several hours or days and are not easily feasible during on-call periods. When it is not possible to obtain compatible RBC units and/or exclude the presence of underlying allo-antibodies, transfusions for these patients are often delayed or even abandoned for fear that the patients will suffer from haemolytic reactions. A common practice in routine transfusion services is to retain the so-called 'least incompatible' RBC units. This term refers to units that are at least ABO-compatible and present the weakest positive results in compatibility tests. Although commonly used in transfusion practice, this is controversial and discouraged due to the residual risk of undetected allo-antibodies [36]. However, several recent studies evaluating the impact of 'least incompatible' transfusions in AIHA have concluded that they were safe and effective [37–39].

Previous recommendations discouraged RBC transfusions in AIHA, stating that transfused RBCs would be destroyed similar to autologous RBCs and that transfusions would therefore be illogical and ineffective [40]. Conversely, recent guidelines recommend that transfusions should not be delayed in life-threatening clinical situations even if pre-transfusion compatibility tests are not fully completed. Nevertheless, the presence of allo-antibodies should be previously excluded, if possible. Patients with no history of pregnancy or transfusion present virtually no risk of allo-immunization, and for these subjects recommendations suggest that ABO-RHD/K matching may be sufficient in an emergency situation [2, 17]. For other subjects, more extended phenotyping is recommended using monoclonal reagents or genotyping [17, 41]. For patients with cold-reactive antibodies, RBC units should be preheated before transfusion using blood warmer devices [2, 17, 40].

Recent paediatric recommendations still suggest, however, to limit the transfusions only to very severe cases of anaemia, associated with a deterioration of vital parameters. In cases where transfusion is absolutely necessary, it is recommended to match the patients' extensive phenotype (RHCE; K; Jka/Jkb; Fya/Fyb; S/s) and to transfuse only the minimum amount required to improve symptomatology [2, 18].

In the particular case of AIHA secondary to allogeneic HSCT, in which AIHA presentation may be severe and life-threatening, it is also recommended that transfusions should not be delayed [16]. RBC units should ideally be compatible with the blood groups of both the HSCT donor and the recipient, but if this not possible, the donor's RBC phenotype should be chosen or, in the particular case of a mixed RBC chimerism, the predominant phenotype.

'IN VIVO' COMPATIBILITY TESTS

A practice known as 'in vivo compatibility testing' has been described in order to determine whether the patient's RBC antibodies are clinically significant [42, 43]. Depending on the methodology, small

amounts of incompatible RBCs labelled with a radioisotope (^{51}Cr) can be injected to the patient and haemolysis assessed by quantifying the radioactivity release in plasma after a few minutes and 1 h post injection. An easier alternative is the rapid injection of a small quantity of a standard RBC unit, followed by a period of observation and optionally a blood sampling in order to quantify the level of free plasma haemoglobin, translating intravascular haemolysis. In the absence of transfusion reaction at the end of the observation period, the test result would be interpreted as 'compatible' and the transfusion could be continued.

The international consensus recommendations suggest that an in vivo compatibility test should be performed for each transfusion in AIHA patients, that is, a rapid transfusion of 20 mL from the RBC unit followed by 20 min of observation [17].

AVAILABLE TECHNIQUES FOR LIMITING AUTO-ANTIBODY INTERFERENCES

Various techniques have been described to limit auto-antibody interference in pre-transfusion testing, aiming at detecting possible underlying allo-antibodies. In this section, we will detail the most frequently described techniques.

SERUM DILUTION

A simple and rapid technique that has been described is the dilution of patient's serum, aiming at the dilution of auto-antibodies in order to reduce interferences and improve detection of underlying alloantibodies, with the assumption that the titre/reactivity of alloantibodies would be higher than that of the auto-antibodies. Although the effectiveness of this technique is very limited because the majority of sera from patients with AIHA remain panreactive after dilution, the procedure may be easily and rapidly performed in laboratories with limited technical and human resources [44]. Nevertheless, this technique carries the risk of false negative results in the presence of low-titre allo-antibodies, which may become undetectable after dilution [45].

ADSORPTION TECHNIQUES

The ideal technique for removing auto-antibodies from the patient's serum is by adsorption onto RBCs prior to the testing [46]. The adsorbing cells can either be the patient's autologous RBCs (i.e., autoadsorptions) or allogeneic RBCs (i.e., allo-adsorptions).

Auto-adsorption, which is illustrated in Figure 1, should be preferred because it removes only auto-antibodies and not allo-antibodies. Unfortunately, auto-adsorptions present some limitations, such as the requirement of a certain amount of RBCs from patients with severe anaemia. Furthermore, this technique cannot be used in case of a recent transfusion history (less than 3–4 months) because circulating allogeneic RBCs might still be present in the patient's blood and adsorb allo-antibodies [47]. Another limitation is that RBCs from AIHA patients are often saturated with auto-antibodies that bind in vivo. In order to allow free serum auto-antibodies to adsorb in vitro, the RBCs first need to be treated. Various techniques have been described to this purpose while preserving cellular integrity [48–52]. ZZAP, a reagent composed of dithiothreitol (DTT) and papain, is effective for dissociating IgG molecules bound to the RBC surface [48]. Proteolytic enzymes and ZZAP treatments denature some antigens of the Duffy, Kell and MNSs systems, making these treatments inappropriate for adsorptions when auto-antibodies are directed against antigens of these systems [53].

When it is impossible to perform auto-adsorption, differential allo-adsorption should be considered. To this end, a minimum of three RBC samples with different phenotypes should be carefully selected in order to avoid adsorption ('loss') of clinically significant alloantibodies during the adsorption cycles. Ideally, RBCs with the most similar phenotype to patient's RBCs should be included, and antigennegative phenotypes for each of the main antigens of the RH, Kell, Duffy, Kidd and MNSs systems must be represented among these RBCs [52, 54]. The implementation of allo-adsorptions is more laborious than auto-adsorptions, as a minimum of three different RBC samples, whose extended phenotypes must be determined, have to be available. Allo-adsorptions as well as residual serum analyses must be performed on different series. An alternative to some phenotypic constraints is the treatment of allogeneic RBCs with proteolytic enzymes, as these treatments denature the antigens of certain systems. Otherwise, RBCs used for allo-adsorptions can remain untreated [33, 45, 52]. Another major disadvantage of alloadsorptions is the risk that allo-antibodies to high-frequency antigens may be adsorbed on all the allogeneic RBC samples and pretransfusion test results may become false negatives.

In order to achieve efficient adsorption more rapidly, the use of potentiating media that promote antigen–antibody reactions has been proposed, notably polyethylene glycol (PEG) or a low ionic strength solution (LISS). The presence of PEG during the adsorption cycles eliminates auto-antibody interference quite efficiently without prior RBC treatment. However, a reduced or even lost detection of allo-antibodies when using PEG has been described, possibly caused by protein precipitation, especially for allo-antibodies displaying weak initial reactivity [55]. LISS is also known to promote antigen–antibody interactions and can also be used for adsorption. The main advantage of LISS is that it allows the use of the gel-filtration technique for residual serum testing, unlike PEG which may cause aspecific agglutination of all RBCs [56–58].

IN VITRO COMPATIBILITY TESTS

The most common classical compatibility test is based on the use of anti-human globulin (IAT), and like other agglutination techniques, this test can be performed using different types of solid substrates, for example, tubes, columns/gel-filtration or microplates. In AIHA, interferences in IAT crossmatches and screening/panels can vary depending on the solid supports and potentiating media that are used. In the current practice of many laboratories, a tube/saline agglutination technique followed by anti-IgG IAT is frequently used for testing in AIHA patients, as it presents less interferences than other solid supports and poly-specific antiglobulins [59].

If interferences persist in compatibility tests, caution is advised especially when reactivity against donor RBC units is more important than the agglutination strength of autologous crossmatch control, potentially indicating the presence of allo-antibody/ies in addition to the auto-antibody.

Some authors question the usefulness of IAT compatibility tests in AIHA compared to a simple 'immediate-spin' crossmatch whose only usefulness is to show ABO group incompatibilities [60]. Other authors suggest a prophylactic approach in these patients by respecting the erythrocyte phenotype or genotype [41, 61], an approach that may also call into question the usefulness of compatibility testing. The optimal method would be to perform auto- or allo-adsorptions before making the IAT crossmatch with residual serum [52].

COLD-REACTIVE ANTIBODIES

Interferences due to cold-reactive auto-antibodies can often be eliminated by working at warm temperatures (37°C) eventually combined with the saline/tube + anti-IgG IAT technique. When it is necessary to work with RBCs from these patients, which usually agglutinate spontaneously at room temperature, washes of the RBCs in saline at 37°C should be performed prior to testing.

A method for removing specifically cold-reactive IgM autoantibodies is the denaturation with 2-mercaptoethanol or DTT. This treatment eliminates IgM interferences and thus allows the detection of possible underlying allo-antibodies, which are usually IgG-type and not affected by these treatments. In some cases, auto-antibody adsorptions at cold temperatures may be necessary, especially when cold-reactive IgG antibodies are involved [62].

DISCUSSION

TRANSFUSION SUPPORT IN AIHA

Although various national guidelines had been published, in the beginning of 2019 there was still a lack of international standardization regarding AIHA terminology, definitions, diagnosis and treatment [1]. The first international recommendations aiming at an harmonization of these different concerns have only recently been published [17]. Since then, the COVID-19 global pandemic made AIHA management a topical issue, as several reports described the occurrence of AIHA associated with SARS-CoV-2 infection [25–27, 29] or vaccination [63, 64].

Except for paediatric cases [2, 18], the various recent guidelines in adults tend to agree that transfusions should not be abandoned or delayed in critical cases of AIHA [2, 17]. Indeed, there is little evidence in the literature for transfusion-induced exacerbation of haemolysis in AIHA patients. Conversely, some studies and case reports rather alert about the risk of clinical decompensation and death when transfusions are too much delayed or abandoned [39, 65]. Lee et al. [60] and Chen et al. [37] observed that transfusions with 'incompatible' RBC units in 222 and 450 AIHA patients, respectively, did not increase the risk of haemolytic transfusion reactions and that they were effective. Park et al. [38] published similar results.

The best approach for choosing RBC units is to prophylactically select the most similar phenotype, as this reduces both the risk of haemolytic transfusion reactions and new allo-immunizations. This requires knowledge of the patient's extensive RBC phenotype, which is not always technically feasible for AIHA patients who present highly positive DATs and/or recent transfusion history [61]. An alternative to circumvent this problem is to perform genotyping of these patients [17, 18, 41]. For urgent transfusion needs, however, it is not always possible to wait for genotyping results.

In future trials, it could be interesting to evaluate the benefit of IVIg administration concomitantly with RBC transfusions, with the purpose of increasing transfusion efficacy by reducing the destruction of RBC. IVIg could neutralize auto-antibodies, thanks to a small proportion of anti-idiotype antibodies [66], which might explain why higher doses of IVIg are necessary for therapeutic effects in autoimmune diseases compared to immunodeficiencies. Unfortunately, the lack of raw materials for IVIg production represents a major limitation. High doses of IVIg might also increase the risk of side effects (allergic reactions, renal failure, thromboembolic reactions, etc.) including the risk of IVIg-related haemolysis

[67], especially in patients with blood groups A/AB. Thus, evaluation of purified anti-idiotype antibodies in AIHA and/or transfusion conflicts will be of interest.

PRE-TRANSFUSION TESTING IN AIHA

The auto-adsorption technique with ZZAP pre-treatment of RBCs appears to be the optimal serological method for detecting alloantibodies underlying auto-antibodies [46, 48, 49, 52]. The main limitation of this technique is that it is time consuming, as RBC treatment and adsorption cycles can already take several hours. Other important limitations arise from the amount of autologous RBCs required for patients with low haematocrit value, and the prerequisite of no recent transfusion history. When auto-adsorption cannot be performed, the best alternative is allo-adsorption, although this does not allow the detection of allo-antibodies directed against high-frequency antigens. Their constraining and time-consuming implementation represents another major disadvantage, limiting the interest of this technique to patients presenting a risk of allo-immunization.

According to several authors [33, 45, 57, 68], adsorption in the presence of PEG would give comparable results to reference adsorption techniques while requiring less time and fewer adsorbing RBCs. In contrast, other publications have shown a reduced detectability or even disappearance of some clinically significant allo-antibodies in the presence of PEG [55, 69, 70].

One hypothesis that could explain these discrepancies in the literature is the storage of the serum–PEG mixture, as PEG is known to cause protein precipitation. Leger et al. [71] confirmed the hypothesis that some allo-antibodies may become less detectable due to precipitation after short-term storage, and therefore they recommend performing IAT on residual serum/PEG on the day of adsorption. Another study concluded that significant precipitation of immunoglobulins already occurs during the adsorption step [70]. The use of LISS in adsorptions could also lead to a decrease in allo-antibody reactivity, probably resulting from a simple dilution effect [56].

Another highly controversial technique is serum dilution. According to Leger and Garratty [45], it displays poor efficiency, as over 70% of sera with auto-antibodies retained panreactivity and 27% of samples containing allo-antibodies gave false negative results. Only in 19% of samples containing allo-antibodies, dilution was able to reveal their presence. Øyen and Angeles [44], who described serum dilution, found a similar proportion of auto-antibody-free samples after dilution, but they did not observe false negative reactions in the presence of allo-antibodies. This technique may be of interest as a first-line test in an urgent transfusion setting, as it is a simple and rapid way of detecting an underlying allo-antibody in approximately 20% of patients. However, the presence of an allo-antibody cannot be excluded based on negative results obtained with this technique.

An important notion to keep in mind is the increased prevalence of allo-antibodies in AIHA subjects, as described in various studies [33, 38, 46, 57, 61, 68], especially in case of transfusion or pregnancy history.

Table 2. Algorithm for laboratory transfusion management in autoimmune haemolytic anaemia.

A: Transfusion emergency (<4 h)	B: Temporarily stable (transfusion in 4–12 h)	C: Non-urgent transfusion (>12 h)
A1. Selection of RBC units • ABO-compatible and RHDCE/K-compatible when available • Compatible with an eventual allo-antibody • Kidd-, Duffy- and Ss-compatible ONLY IF patient pheno/genotype and RBC units available in time	**B1. Selection of RBC units** • ABO-compatible and RHDCE/K-compatible when available • Compatible with an eventual allo-antibody • Kidd-, Duffy- and Ss-compatible ONLY IF patient pheno/genotype and RBC units available in time	**C1. Selection of RBC units** • ABO-compatible and RHDCE/K-compatible when available • Compatible with an eventual allo-antibody • Kidd-, Duffy- and Ss-compatible (if possible)
A2. Minimal pre-transfusion tests • 'Least incompatible' units in compatibility tests (IAT gel-filtration technique)	**B2. Minimal pre-transfusion tests** • Warm-type AIHA: serum dilution → IAT screen/panel • Cold-type AIHA: DTT serum-treatment → IAT • Tube technique (saline + anti-IgG IAT) → compatibility tests + RBC panel (if risk of allo-immunization)	**C2. Minimal pre-transfusion tests** • Warm-type AIHA: serum dilution → IAT screen/panel • Cold-type AIHA: DTT serum-treatment → IAT • Tube technique (saline + anti-IgG IAT) → compatibility tests + RBC panel (if risk of allo-immunization) And in addition • Patient RBC elution (possible identification of auto- or allo-antibodies bound to patient RBCs)
A3. Optional pre-transfusion tests (if time) • Warm-type AIHA: serum dilution → IAT screen/panel • Cold-type AIHA: DTT serum-treatment → IAT • Tube technique (saline + anti-IgG IAT) → compatibility tests + RBC panel (if risk of allo-immunization)	**B3. Optional pre-transfusion tests (if time)** • Patient RBC elution (possible identification of auto- or allo-antibodies bound to patient RBCs)	**C3. Optional pre-transfusion tests (if risk of allo-immunization)** • Auto-adsorptions on pre-treated RBCs (if possible) Or • Differential allo-adsorptions on native RBCs
+ 'In vivo' *crossmatch*	+ 'In vivo' *crossmatch*	+ 'In vivo' *crossmatch*

Note: Depending on the degree of transfusion emergency (A, B or C), a standardized approach is proposed for the selection of RBC units (A1 → C1), minimal pre-transfusion tests that should be performed (A2 → C2) and optional pre-transfusion tests, depending on time for analyses and/or patient history (A3 → C3).

Abbreviations: AIHA, autoimmune haemolytic anaemia; DAT, direct antiglobulin test; DTT, dithiothreitol; IAT, indirect antiglobulin test (refers to gelfiltration IAT tests, except for tube technique cited in A3, B2 and C2); RBC, red blood cell.

CONCLUSIONS

The general answer to the question 'when to transfuse patients with AIHA?' could be that they should be transfused as soon as possible in cases of severe and life-threatening anaemia. Except for paediatric cases where transfusions are recommended only if vital signs are impaired, the most recent data for adults show a benefit of prompt transfusion management. It is difficult to define universal transfusion thresholds for these patients, as the decision must be assessed on a case-by-case basis depending on possible co-morbidities and the evolution of the disease/AIHA. Furthermore, given the risk of rapid clinical decompensation on increased haemolysis when auto-antibodies reach very high tires, haemoglobin monitoring needs repeated measurements because its concentration can decrease very rapidly.

The answer to the question 'how to transfuse patients with AIHA?' is more complex, as all pre-analytical techniques have their limitations, whether through the risk of non-detection of an alloantibody or their laborious and time-consuming implementation. An important factor that needs

to be considered is the degree of transfusion emergency. When facing an urgent situation, the best approach seems to be the preventive selection of the most compatible RBC phenotype available for transfusion units, which can be combined with the results of minimum laboratory analyses by choosing 'least incompatible' units and/or performing antibody screening on diluted serum. Although highly controversial, these approaches are easily and quickly performed in all immuno-haematology laboratories, and as a matter of common sense it seems preferable to include these results in the selection process rather than perform no laboratory testing at all when transfusion should not be delayed. In situations where transfusion can be delayed by a few hours/days, more thorough immunohaematological testing should be performed, especially for patients presenting a risk of allo-immunization. An algorithm for transfusion management is proposed in Table 2. The prerequisite is close communication between laboratory and medical staff. In all cases, in vivo compatibility assessments as recommended [17] should be performed at the patient's bed for each unit transfused.

A decisional algorithm for the selection of RBC units in patients with warm AIHA was recently published [35]. In the algorithm for transfusion management that we describe (Table 2), in addition to RBC selection we also focus on pre-transfusion laboratory tests that should be performed, depending on the degree of emergency required for transfusion support and on type of AIHA. The proposed algorithm in this review further takes into account the recommendations for transfusion management from the international consensus meeting [17].

In conclusion, it appears that transfusion management of patients with AIHA is often severely delayed or avoided due to concern about possible haemolytic transfusion reactions. However, recent data and guidelines suggest that the main risk for AIHA patients arises from delayed transfusion management in lifethreatening situations. Although it is difficult to define a standardized approach given the diversity of presentations, there should be awareness about the risk represented by a transfusion delay in adult AIHA patients.

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The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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