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ORIGINAL RESEARCH

Resolving Incompatible Blood Cross-Matching: The Role of 37°C Water Bath in Transfusion Safety

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Background: Incompatible cross-matching at room temperature can delay critical blood transfusions. We report that red cells from patients requiring blood transfusion agglutinated after cross-matching at room temperature, but changed from agglutination to dispersion after a water bath at 37°C. The aim was to investigate whether re-cross-matching at 37°C is necessary for some patients with incompatible cross-matching.

Methods: This prospective observational study analysed all incompatible cross-matched blood samples at the Blood Transfusion Department of Chifeng Municipal Hospital from 1 May 2024 to 31 July 2024. Cross-matching was performed using a fully automated system and the Polybrene method. For incompatible samples, the Polybrene method was repeated after the samples were placed in a 37°C water bath for 5 minutes.

Results: Thirty patients with cross-match incompatibilities were included. Among these, agglutination was reversed after the 37°C water bath in 28 patients, indicating compatibility. Only 2 patients had true blood group incompatibility. We detail the results of one representative patient from the 28 with reversed agglutination. This patient showed agglutination using the fully automated device and the Polybrene method, but not with the saline method. After a 37°C water bath, the agglutinated erythrocytes dispersed into a single-cell state upon microscopic examination.

Conclusion: For selected patients, cross-matching at 37°C can resolve false-positive agglutination, ensuring transfusion safety and timely access to blood products. This simple method can be integrated into the clinical cross-matching workflow.

Plain Language Summary:

(1) Some patients experience incompatible blood cross-match results at room temperature, which can delay lifesaving transfusions.
(2) We found that for most patients in our study, this incompatibility was resolved by warming the blood sample to 37°C, allowing the transfusions to proceed safely.
(3) Incorporating a 37°C cross-matching step can help ensure that patients receive needed blood transfusions without unnecessary delay.

Keywords: cross-matching incompatibilities, polybrene method, red cell aggregation, irregular antibodies, 37°C water bath

Introduction

Cross-matching is a serological test used to determine the compatibility of blood between donors and recipients to ensure the safety of blood transfusion.[1] Pre-transfusion testing aims to achieve safe transfusion effects by providing the best blood products

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without causing harm to the patient's body. Many serological tests are routinely performed in the transfusion department, such as ABO/Rh typing, antibody screening or identification, and cross-matching technology, to identify blood groups and unexpected antibodies present in the blood in a timely and accurate manner. In most cases, these testing technologies can successfully match compatible blood, but there are still some challenges. For example, the serum of patients with autoantibodies agglutinates with normal red blood cells (RBCs). In fact, some patients may not be able to obtain a qualified cross-match for a long time in order to transfuse RBCs in time, which affects the treatment of the disease.[2] Therefore, such complex cross-matching of incompatible patients has caused a lot of trouble for clinicians and transfusion physicians.

However, a subset of these incompatibilities may be due to temperature-sensitive antibodies (eg, cold agglutinins) or non-specific reactions rather than true antigen-antibody mismatches. This can lead to the unnecessary withholding of blood products. Therefore, this study was designed to evaluate whether a 37°C water bath incubation could resolve cross-match incompatibilities observed at room temperature, particularly in patients with hematological disorders requiring frequent transfusion, thereby facilitating timely and safe transfusion therapy.

In theory, the agglutination reaction of red blood cells caused by cross-matching is an antigen-antibody reaction in the body.[3] The antigen-antibody reaction is a reversible chemical reaction whose force is a weak bond, and the formation of the red blood cell antigen-antibody complex must involve two or more weak bonds. Many factors (such as temperature, pH, ionic strength and enzyme treatment of the red cells, etc) affect the antigen-antibody reaction.[4] RBC antibodies are traditionally divided into "cold" and "hot" types. The most appropriate temperature for the antigen-antibody reaction may depend on the chemical properties of the epitope and paratope, and more specifically on the type of weak bonds involved. Hydrogen bonds are more stable at low temperatures, and the strength of hydrophobic bonds increases with temperature. Even for weak antibodies, the antigen-antibody reaction can be stabilised at low temperatures.[5]

Given the complexity of the body's immune system, we report a study in which the red blood cells of patients with clinical transfusion needs who were cross-matched at room temperature and whose serum reaction tubes were returned to a 37 °C water bath could change from an agglutinated state to a dispersed state. This study aims to investigate the need for re-crossmatching at 37°C in some patients with crossmatch incompatibilities.

Materials and Methods

Study Design and Population

This retrospective observational study analyzed all cases of initial incompatible blood cross-matching identified in the blood transfusion department of Chifeng Municipal Hospital between 1 May 2024 and 31 July 2024 (3 months). The study population consisted of patients requiring transfusion who exhibited incompatible cross-matching results during the study period. A total of 30 clinical cases were collected. All cases were from patients in the haematology department who had undergone long-term repeated blood transfusions.

Blood Sample Preparation

All requests for blood components must be accompanied by a completed and authorised blood request form designed by the Chifeng Municipal Hospital Blood Transfusion Committee. Collect 3 mL of fresh whole blood in an ethylenediaminetetraacetic acid (EDTA) anticoagulant sample tube and attach a label with relevant information (hospital number, name, age, department, bed number and unique sample identification barcode, etc) to the tube. Centrifuge at 3000 × g for 3 minutes to separate red cells and serum/plasma. If blood is transfused again more than 3 days after cross-matching, a new blood sample must be collected.

Blood Typing and Cross-Matching

Blood typing and cross-matching were performed using the ORTHO AutoVue Innova fully automated blood typing and cross-matching system (Johnson & Johnson, USA). Manual cross-matching was performed using the Polybrene method (BASO, China). Take two test tubes, mark them for the major cross-match (patient serum vs donor red blood cells) and minor cross-match (donor serum vs patient red blood cells). Add 2 drops of patient serum to the primary tube and 1 drop of 3–5% donor erythrocyte suspension to the secondary tube, and vice versa. Use a centrifuge (Xiangyi Company, China) at

Table 1 Characteristics of Patients with Cross-Match Incompatibility (n=30)

Characteristic	Value
Median Age (range)	62 years (24–87)
Gender (Male/Female)	15 / 15
Primary Diagnosis	
- Anemia	11
- Acute Monocytic Leukemia (AML)	1
- Multiple myeloma (MM)	6
- Myelodysplastic Syndrome (MDS)	1
- Primary myelofibrosis (PMF)	2
- Other Hematological Disorders	9
Patients with a history of blood transfusion	14

3400 rpm, centrifuge for 10s, and observe with the naked eye for agglutination (this step is called the saline method). Then gently mix the suspension, add 0.7mL of LIM to each, then add 2 drops of polybrene solution to each and mix well. Centrifuge at 3400 rpm for 10 seconds, discard the supernatant and leave approximately 0.1 mL of liquid at the bottom of the tube. Shake the tube gently and observe visually for agglutination of the red cells. If there is no agglutination, repeat the procedure. Finally, add 2 drops of resuspension solution, swirl gently to mix and observe the results. If the agglutination disperses within 60 seconds, it is a non-specific agglutination caused by polybrene and the blood matching result is consistent; if the agglutination does not disperse, it is a specific reaction of erythrocyte antigen-antibody binding and the blood matching result is inconsistent. You can also pour it on a microscope slide and observe it under the microscope.

Data Analysis

Data analysis was primarily descriptive, focusing on the frequency of agglutination reversal after the 37°C water bath intervention.

Results

We collected 30 cases of patients with cross-match incompatibility in the hospital during the last three months. Table 1 summarizes the characteristics of these patients. Both the polybrene method and the instrument method were used as primary and secondary tests for all agglutinations. The polycondensate method was used to re-match the above incompatible samples and the samples were placed in a 37°C water bath for 5 minutes. After the 37°C incubation, agglutination was reversed in 28 out of the 30 patients (93.3%), indicating compatibility. Only 2 patients (6.7%) had true blood group incompatibility (Figure 1A). We show the cross-matching results of one representative patient from the 28 with reversed agglutination. This patient showed agglutination in both the primary and secondary tests after cross-matching using a fully automated device (Figure 1B). The subsequent saline method showed no agglutination (Figure 1C). When the polybrene method was used again, significant agglutination occurred in both the major and minor tests (Figure 1D). The test tube was then placed in a 37°C water bath for 5 minutes and the agglutinated red cells were re-isolated (Figure 1E). Microscopic observation also showed that agglutinated red blood cells were transformed into single red blood cells after a 37°C water bath (Figure 1F).

For the 28 patients with reversed agglutination, blood was transfused after clinical consultation. The blood was administered slowly. No acute adverse reactions occurred during transfusion, and no delayed adverse reactions were observed 24 hours after transfusion.

Discussion

Cross-matching is the final step in ensuring the safety of blood before transfusion. In this test, the donor's red blood cells and serum interact with the patient's red blood cells and serum to check for red cell agglutination.[6] Agglutination indicates blood incompatibility and is the final guarantee of blood transfusion safety. A common problem encountered by

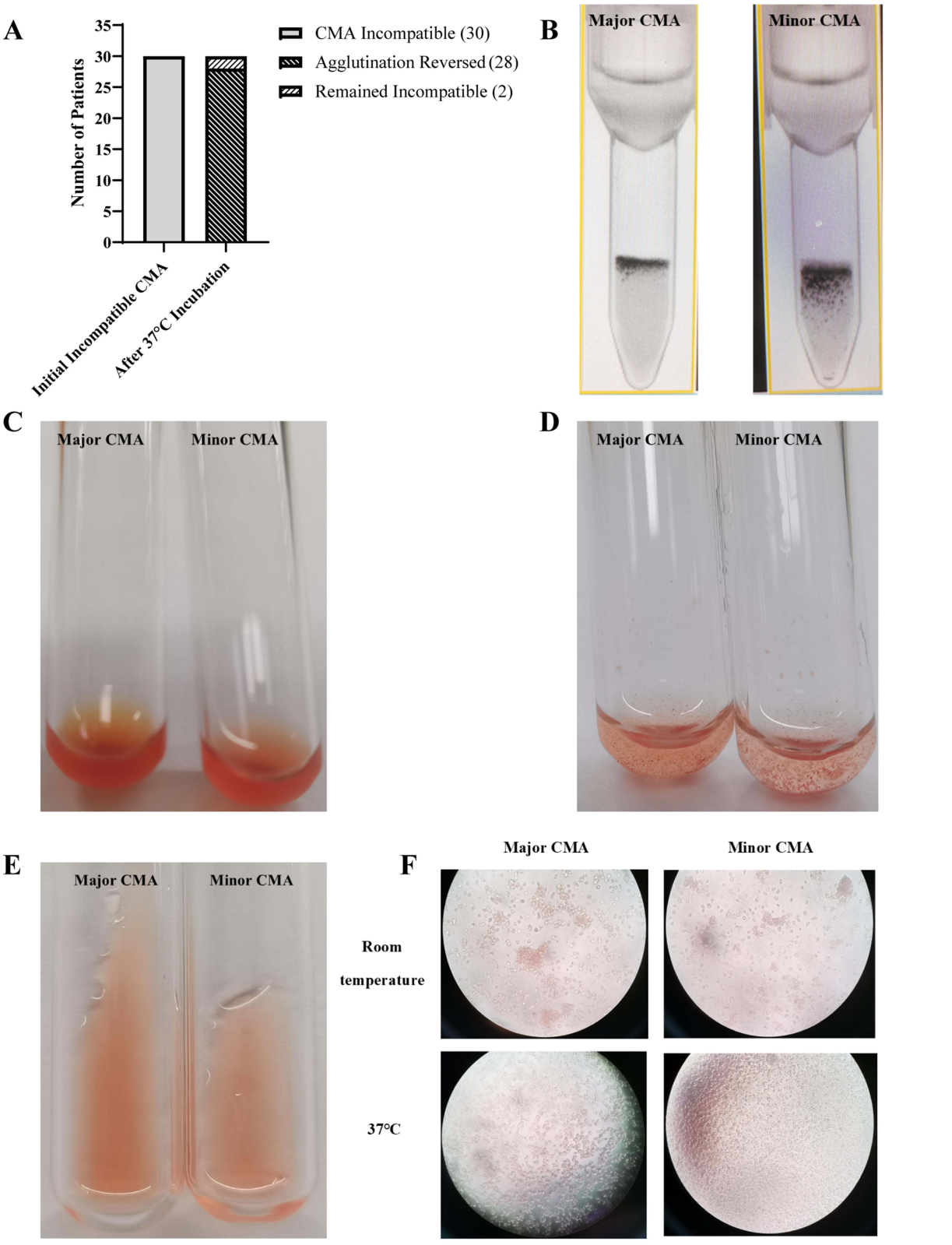

Figure 1 The 37°C water bath allows cross-matching incompatible red blood cells to redisperse into a single-cell state. (**A**) Summary of results: 30 patients with initial cross-matching incompatibility were re-tested after a 37°C water bath; agglutination was reversed in 28 patients. (**B**) cross-matching was performed using a fully automatic instrument (showing agglutination). (**C**) Cross-matching was performed using the saline method (no agglutination). (**D**) Cross-matching was performed using the polybrene method (showing agglutination). (**E**) The agglutinated tube from (**D**) was placed in a 37°C water bath for 5 minutes (showing dispersion). (**F**) Microscopic observation (40x magnification) of red blood cell aggregation before and after the 37°C water bath following the polybrene method. The major cross-match (patient serum vs donor red blood cells) and minor cross-match (donor serum vs patient red blood cells) are shown.

Abbreviation: CMA, Cross-Match.

haematologists in transfusion is cross-match incompatibility. Most blood banks can only perform type and screen and cross-matching analysis. Our research group has found that the likelihood of cross-match incompatibility can be reduced by observing the results of some cross-matches after a 37°C water bath.

The body's immune system produces five types of immunoglobulin: IgM, IgD, IgG, IgA and IgE. Each immunoglobulin has a unique effector function.[7] There are more than 40 blood group systems and more than 300 red cell antigens. ABO system antigens and Rh D antigens are the most immunogenic antigens. Anti-A and anti-B are naturally occurring antibodies of the IgM type, whereas anti-D antibodies are of the IgG type.[8] The saline method usually detects IgM antibodies, which are often amplified at 4°C.[9] Irregular antibodies generally refer to other blood group related antibodies in the serum other than anti-A and anti-B.[10] The polycondensation method has high sensitivity and can accurately detect low concentrations of irregular antibodies, mainly for the detection of IgG antibodies.[11] Therefore, our study found that cross-matching incompatibility often occurred in the coagulation amine method, while there was no obvious agglutination in the saline method. This also shows that patients who receive long-term blood transfusions produce unpredictable irregular antibodies in their blood.

Almost all people with type O blood spontaneously develop anti-A and anti-B alloantibodies, but the mechanisms of alloimmunisation induced by red blood cells after transfusion are complex. Some patients, known as non-responders, do not develop alloantibodies despite multiple red cell transfusions. Conversely, some patients develop alloantibodies after previous exposure to red cell alloantigens.[12] It is unclear whether non-responders fail to develop an immune response to allogeneic red cells or whether some people develop alloantibodies after transfusion but are not detected. The underlying disease state of the patient at the time of transfusion may influence the likelihood of red cell alloimmunisation. Patients with sickle cell disease, myelodysplastic syndrome, hereditary telangiectasia, systemic lupus erythematosus and rheumatoid arthritis are associated with an increased risk of alloimmunisation, such as altered immune activation.[13] In contrast, patients with conditions that can impair immune function, such as bone marrow failure syndrome, acute myeloid or lymphocytic leukaemia, or chronic hepatorenal insufficiency, are theorized to be less likely to develop alloantibodies after red cell transfusion.[14–16] Most patients with cross-match incompatibility in our hospital transfusion department come from the haematology department, usually with various types of haematological diseases. According to the above theory, such patients are theorized to be unlikely to produce antibodies against red blood cells. Our research results also show that in some patients with cross-matching incompatibility, the aggregated red blood cells disintegrate into single cells after the polybrene reaction is placed in a 37°C water bath. This may indirectly suggest that cross-match incompatibility does not necessarily mean that the patient's body has produced antibodies against red cells. There may be other reasons for false positive results and the specific mechanism remains to be investigated.

Antibodies to red blood cells are traditionally classified as "warm" and "cold", with warm antibodies referring to autoantibodies that react optimally with red blood cells at 35–40°C. Cold antibodies react optimally at temperatures below 30°C, especially below 4°C. This is related to the optimal thermal temperature of the antigen-antibody reaction.[17] In the early 20th century, Landsteiner discovered that blood clotted at low temperatures. Horstmann and Tatlock confirmed these findings and detected cold agglutinins in the sera of patients with primary atypical pneumonia.[18] A few years later, Schubothe coined the term "cold agglutinin disease". Cold agglutinins react at temperatures of 0–4°C and can cause agglutination in the patient's nose, ears or fingers, leading to painful cold-induced symptoms.[19] As research progresses, it is likely that the optimal agglutination temperature depends on the chemical properties of the epitope and paratope (type of weak bonds). Hydrogen bonds are exothermic and more stable at low temperatures, and the strength of hydrophobic bonds increases with temperature. Even for warm antibodies, antigen-antibody reactions are expected to stabilise at low temperatures.[20] Therefore, we placed the polybrene test mixture at 37°C to affect the reaction temperature of the antigen and antibody, causing some of the agglutinated red blood cells to deaggregate, which is consistent with the behavior of cold agglutinins.

Our study also has some limitations. First, we did not strictly record the room temperature during the cross-matching process, so we ignored the effect of room temperature on the experimental results. Second, due to limited case resources in the hospital, we selected haematology patients as research subjects, which may limit the generalizability of our findings to other patient populations. Finally, due to the limitations of the experimental conditions, we could not conduct in-depth research on the mechanism of this phenomenon, such as identifying the specific type of antibodies involved.

In conclusion, our study found that cross-matching with the polybrene method followed by a 37°C water bath can convert partially agglutinated red blood cells into single cells in a significant proportion of patients with initial incompatibility. This simple technique can help meet the safety requirements of clinical blood transfusion by reducing false-positive incompatibility results, potentially preventing delays in transfusion therapy for patients. Our study provides preliminary evidence and inspiration for further research into the methodology of fully automated blood-matching devices and the improvement of clinical cross-matching workflows, particularly in settings where sophisticated antibody identification techniques are not readily available.

Conclusions

In conclusion, we have found in our clinical practice that in some patients who require blood transfusion, red cell agglutination occurs during crossmatching at room temperature. Repeating the crossmatch test with a 37°C incubation step demonstrated that the agglutinated red cells could be re-dispersed in the majority of patients (28/30), indicating a false-positive result. This evidence suggests that in clinical transfusion practice, particularly for patients with hematological conditions, incorporating a 37°C cross-matching step could be a valuable procedure to ensure that patients have timely access to essential transfusion therapy while maintaining safety.

Data Sharing Statement

The data generated in the present study may be requested from the corresponding author.

Ethical Consideration

This study was reviewed and approved by the Ethics Committee of Chifeng Municipal Hospital. This subject strictly abides by the Declaration of Helsinki, and all patients were informed and agreed to participate in this project study, and the red blood cells from the blood donors were all from the Chifeng Central Blood Station and passed the test.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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