EDUCATIONAL COMMENTARY – INVESTIGATION OF IMMEDIATE TRANSFUSION REACTIONS

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**Learning Objectives**

On completion of this exercise, the participant should be able to

- discuss the laboratory tests required by the AABB Standards for Blood Banks and Transfusion Services (AABB) and the College of American Pathologists (CAP) Transfusion Medicine Checklist to investigate an acute hemolytic transfusion reaction (AHTR);
- propose additional laboratory tests to confirm or exclude a specific transfusion reaction diagnosis; and
- correlate the transfusion recipient’s clinical symptoms, component transfused, and laboratory test results to identify the type of transfusion reaction experienced by the recipient.

There are numerous types of transfusion reactions of varying degrees of severity, with some sharing common symptoms. To diagnose the type of transfusion reaction a recipient has experienced, the pathologist must consider the recipient’s symptoms (including the timing of symptoms in relation to the length of transfusion), the blood component transfused, and the results of key laboratory tests.

Immediate transfusion reactions (also known as *acute transfusion reactions*) generally develop during the transfusion of a blood component or within 1 to 2 hours following transfusion. This is in contrast, *delayed transfusion reactions* which can occur within several days up to 2 weeks post transfusion. In the text below, the basic steps required by the AABB Standards for Blood Banks and Transfusion Services (AABB) for investigating a suspected immediate transfusion reaction and laboratory tests that differentiate an acute hemolytic transfusion reaction from other types of immediate transfusion. Select case studies will demonstrate how the transfusion recipient’s symptoms, the component transfused, and results of key laboratory tests are correlated to form a diagnosis.

**Initial Investigation**

Investigation into a potential immediate transfusion reaction begins with gathering information from the transfusionist. When a transfusion reaction is first suspected, the transfusionist must immediately stop the transfusion. The patient’s physician should be notified to obtain orders for immediate clinical care and the transfusion service should be contacted for a laboratory transfusion reaction investigation. The
transfusionist should maintain an intravenous (IV) line with saline to facilitate administration of medications or additional blood components that may be required for treatment. The transfusionist must perform a clerical recheck between the patient and the component, verifying the information on the recipient’s identification band against the recipient information on the blood component tag or label; two unique patient identifiers (i.e., name, medical record number, date of birth, and/or blood bank identification band number) must be in agreement. In addition, the donor information on the tag must agree with the unit label, including the donor identification number, ABO/Rh type, and expiration. Clerical errors involving recipient, specimen, or donor misidentification are the primary causes of an acute hemolytic transfusion reaction in which the recipient has a complement-activating antibody directed against donor red blood cell (RBC) antigens.

The transfusionist must also notify the transfusion service of the suspected reaction, unless the only symptom is hives, indicating a mild allergic reaction. The remaining component, along with all associated tubing and infused IV solutions, must be returned to the transfusion service. A post-transfusion specimen must be collected from the recipient, preferably in EDTA, and care should be taken to avoid a traumatic draw that could cause hemolysis of the RBCs. Documentation of the reaction, including the recipient’s symptoms, length of transfusion and volume transfused, should be provided to the transfusion service and recorded in the recipient’s medical record.

Both the AABB Standards and the CAP Transfusion Medicine Checklist require that the transfusion service’s initial investigation of a potential transfusion reaction involve steps to confirm an acute hemolytic transfusion reaction. Acute hemolytic transfusion reactions are caused by the recipient’s own preformed RBC antibodies destroying donor RBCs that possess the corresponding antigen. The patient experiencing an acute hemolytic transfusion reaction will most commonly present with fever, chills, and hypotension. Pain at the site of the IV, in the chest, or lower back; nausea; dyspnea; and/or a feeling of anxiety may accompany these symptoms.

The first step in the transfusion service’s investigation is to repeat the clerical check of recipient and donor information. Historical records of the recipient’s ABO/Rh type and the presence of clinically significant antibodies are compared with the results of pre-transfusion compatibility testing. Compliance with any necessary additional component attributes, such as antigen-negative, leukoreduced, or plasma-reduced, is verified. The post-transfusion reaction specimen is examined for hemolysis of RBCs, a sign of potential complement activation resulting in RBC destruction. The post-transfusion specimen may be compared with the pre-transfusion specimen; hemolysis in the post-transfusion specimen not also
observed in the pre-transfusion specimen is a significant finding, suggesting an immediate acute hemolytic transfusion reaction has occurred.

Before notifying the transfusion service medical director or the recipient’s physician of these results, transfusion service staff should attempt to rule out other causes of hemolysis in the post-transfusion specimen. Other causes may include difficulty drawing the post-transfusion specimen, transfusion of RBCs with an improper IV solution (one other than normal saline), improper storage of the RBC unit (cooled below 1°C or warmed above 42°C), or mechanical lysis of the transfused RBCs (use of a small-gauge needle, use of an infusion pump not approved for transfusions). If there is a delay in collecting the post-transfusion specimen, icterus may be present due to the accumulation of unconjugated bilirubin, a by-product of RBC destruction.

Next, a determination of the ABO grouping must be performed on the post-transfusion specimen and the results compared with those of the pre-transfusion specimen. Reactions should be carefully observed, looking for mixed-field reactions that indicate the presence of two RBC populations of differing ABO groups. Mixed-field agglutination in a test tube appears as agglutinates floating in a cloudy background. Microscopic observation reveals both clusters of agglutinated RBCs and free floating individual RBCs. In a gel test system, there can be an obvious separation of the two RBC populations, with the agglutinated RBCs trapped within the gel and the free RBCs found at the bottom of the microtube. If the results of the ABO testing on the post-transfusion specimen reveal the presence of incompatible RBCs, the diagnosis of an acute hemolytic transfusion reaction can be confirmed.

The final test required to determine if an acute hemolytic transfusion reaction has occurred is a direct antiglobulin test (DAT) performed on the post-transfusion specimen. In an acute hemolytic transfusion reaction, the incompatible donor RBCs become coated with antibodies present in the recipient’s plasma, yielding a positive DAT. The recipient’s own antigen-negative RBCs do not agglutinate; mixed-field reactions are common. If the DAT is positive in the post-transfusion specimen, a DAT should be performed on the pre-transfusion specimen for comparison. A positive DAT in the post-transfusion specimen is a less significant finding if the pre-transfusion specimen is also DAT-positive. If the post-transfusion DAT is positive using polyspecific antihuman globulin reagent (AHG), testing should be repeated using monospecific anti-IgG and anti-complement AHG reagents. If the DAT is positive with the anti-IgG reagent, an eluate should be prepared from the post-transfusion specimen’s RBCs and tested to determine the specificity of the antibody coating the donor RBCs. It is not uncommon for both the anti-IgG and anti-complement AHG reagents to yield positive DAT results.
A negative DAT does not exclude an acute hemolytic transfusion reaction: the recipient’s antibody may have destroyed all of the donor RBCs. If you see hemolysis in the post transfusion specimen and cannot explain it with non-immune causes, an acute hemolytic transfusion should still be considered, even though the DAT is negative.

Additional (Optional) Testing

Additional laboratory tests may be performed to monitor the course of an immediate hemolytic transfusion reaction. There are several laboratory values that change including hemoglobin, haptoglobin, and lactate dehydrogenase (LDH). Due to the intravascular hemolysis that occurs in an acute hemolytic transfusion reaction, RBC lysis results in an increase in plasma free hemoglobin. Haptoglobin, a parameter that decreases during hemolysis, is an α2-globulin that binds free hemoglobin. However, the plasma free hemoglobin level may increase beyond haptoglobin’s capacity/concentration to bind it. Once the plasma free hemoglobin level reaches 25 mg/dL, free hemoglobin appears in the urine. Some transfusion service protocols include collection of the first urine voided after the transfusion reaction as part of their work-up. Hemoglobin in the urine will yield a positive test for blood and protein on a urine dipstick, but a microscopic examination of urine sediment will be negative for intact RBCs. Levels of unconjugated bilirubin in the plasma will also increase due to the increased RBC destruction, peaking 6 to 7 hours after transfusion, as the liver cannot keep pace with conjugating and excreting this substance. Coagulation tests and platelet counts can be used to monitor the recipient who goes into disseminated intravascular coagulation (DIC). The prothrombin time (PT) and activated partial thromboplastin time (aPTT) will be prolonged, while the fibrinogen level and platelet count will be decreased. The recipient will test positive for fibrin degradation products, and a D-dimer test will be positive.

If the initial investigation reveals a probable acute hemolytic transfusion reaction that cannot be attributed to ABO incompatibility, additional testing must be performed to determine the specificity of the antibody that triggered the reaction, as antigen-negative RBC units must be provided for future transfusions. The antibody screen may be performed on the post-transfusion specimen, although it may take 48 to 72 hours for the antibody titer to increase to detectable levels. The antibody screen and crossmatch may be repeated on the pre-transfusion specimen to determine if there was a technical error that resulted in the antibody going undetected.

Common symptoms of an acute hemolytic transfusion reaction include fever (increase in temperature above normal by at least 1°C), chills, and hypotension. These symptoms may also be seen in transfusion-related acute lung injury (TRALI), febrile non-hemolytic transfusion reactions (FNHTRs), and transfusion-related sepsis. If the initial investigation into the transfusion reaction refutes an acute
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Hemolytic transfusion reaction, further clinical information as well as laboratory testing to confirm or exclude these other reactions should be performed.

According to the US Food and Drug Administration (FDA) figures from 2013, TRALI is the leading cause of transfusion-related death. In addition to fever, chills, and hypotension, TRALI presents with respiratory symptoms including dyspnea and cyanosis. Symptoms generally appear 1 to 2 hours following completion of the transfusion, and plasma and platelet products are implicated more frequently than RBCs. The diagnosis of TRALI is confirmed by a chest x-ray demonstrating bilateral pulmonary infiltrates in addition to other diagnostic criteria established by various consensus conferences. Laboratory testing focuses on determining the presence of leukocyte antibodies in the donor is tested; donors with such antibodies are deferred from future plasma donations.

Transfusion-related sepsis is characterized by a sudden increase in temperature (often by 2°C or more), chills, and hypotension during transfusion. Because they are stored at room temperature, platelet products are implicated more frequently than other components. A Gram stain and culture on the remaining product along with blood cultures on the recipient can provide the diagnosis.

Febrile non-hemolytic transfusion reactions are essentially diagnosed by excluding all other causes for fever and chills experienced during a transfusion or within a few hours of completion of transfusion. FNHTRs are the result of cytokine release in the recipient, which may be triggered by recipient anti-leukocyte antibodies [most frequently HLA antibodies] reacting with donor leukocytes or may be the response to cytokines present in a cellular component (most frequently associated with platelet components). Filtration of cellular components prior to storage helps to reduce the frequency of these reactions by removing leukocytes and preventing the buildup of cytokines.

Anaphylactic transfusion reactions are seen within the first few minutes of transfusion, usually with plasma-containing products. Respiratory symptoms (cough, shortness of breath, bronchospasm) and severe hypotension without fever are hallmarks of these reactions. Transfusion recipients who are IgA-deficient and who have developed anti-IgA are likely to experience an anaphylactic reaction when receiving blood components that contain plasma. To confirm these reactions, the AABB recommends assaying quantitative IgA levels in the recipient's serum along with tests to detect IgA antibodies.

Other immediate transfusion reactions (e.g. allergic reactions, transfusion-associated circulatory overload (TACO), and hypothermia) are diagnosed based on the recipient's symptoms, component transfused, and
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timing of the reaction in relation to the length of transfusion. No specific laboratory testing is defined for these reactions.

Case A
Patient John Jacobs had 6 units of leukocyte-reduced RBCs stored in a refrigerator near the operating room where he was undergoing a liver transplant. The patient’s father, James Jacobs was the liver donor, and also had 2 units of leukocyte-reduced RBCs stored in the same refrigerator. During surgery, John required transfusion with a unit of RBCs. Two RBC units were retrieved from the refrigerator; one for immediate transfusion, and one “just in case.” The second unit remained at room temperature in the operating room for 90 minutes before being transfused. Within 7 minutes of starting transfusion of the second unit, John’s blood pressure fell from 110/84 to 82/50, his temperature increased to 38.5°C (from a pre-op temperature of 37°C), and red urine was noted in his catheter bag. John’s bleeding became increasingly difficult to control, with oozing noted in the surgical field, indicating the possibility of DIC.

Did John experience an immediate transfusion reaction? If so, which type of transfusion reaction is most likely?

The symptoms exhibited by the patient in this case are typical of an acute hemolytic transfusion reaction. However, transfusion-related sepsis should also be considered, as the second RBC unit was left at room temperature for an extended period of time, potentially resulting in bacterial growth. A clerical check soon revealed the problem: the second unit was one of the two units prepared for the patient’s father, James Jacobs. James is group A, RhD-positive. John’s pre-transfusion specimen typed as group O, RhD-positive; his post-transfusion specimen exhibited mixed-field reactivity with anti-A, indicating the presence of both group O and group A RBCs. John’s post-transfusion reaction DAT was positive with both monospecific anti-IgG and anti-C3 AHG reagents, with anti-A identified in the eluate. Given the patient’s symptoms and blood bank work-up, this was an acute hemolytic transfusion reaction due to anti-A, caused by patient misidentification. Had there been no clerical errors, no evidence of ABO incompatibility and a negative DAT, transfusion-related sepsis would be on the differential diagnosis for which a Gram stain and culture of the second RBC unit along with blood cultures on John Jacobs would have been in order.
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(cont.)

Case B
Approximately 1 hour following transfusion with an apheresis platelet unit, a patient exhibited an increase in temperature from 36.5°C to 37.8°C, along with shortness of breath, chills, and a decrease in blood pressure.

What types of immediate transfusion reactions should be considered in this case? What additional testing could confirm or exclude each reaction?

With transfusion of a plasma-containing product, one should consider an anaphylactic reaction or TRALI. Because this was a platelet transfusion, transfusion-related sepsis should also be considered in this case.

The length of time to symptom onset and the presence of fever makes anaphylactic reaction the least likely of the three. Further testing for IgA levels and the presence of anti-IgA would not be indicated at this point. Transfusion-related sepsis generally has a more immediate onset as well, but a Gram stain and culture of the unit along with blood cultures on the patient would be helpful to rule out sepsis as a potential cause. Based on the timing, TRALI is the most likely transfusion reaction. The appearance of bilateral pulmonary infiltrates on a chest x-ray will be helpful in confirming this diagnosis. This reaction should be reported to the donor collection center, which would in turn test the donor for anti-HLA and neutrophil antibodies.

Conclusion
Despite improvements in pre-transfusion testing and precautions to avoid recipient misidentification, immediate transfusion reactions continue to occur. Clerical checks along with ABO and DAT determinations on the post-transfusion specimen can establish that an acute hemolytic transfusion reaction has occurred. Additional laboratory tests monitor the progression of the transfusion reaction. If an acute hemolytic transfusion reaction is eliminated as a potential diagnosis, evaluation of the recipient’s symptoms and consideration of the component transfused and length of transfusion along with additional confirmatory tests can establish the transfusion-reaction diagnosis and allow the physician to provide proper, timely treatment.

References


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