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TITLE: Blood Bank Evaluation of Autoimmune Hemolytic Anemia

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Hello, my name is Dr. Eric Gehrie. I am an assistant professor of pathology and associate director of the transfusion medicine division at Johns Hopkins.

Today, I am pleased to have a chance to talk with you about the blood bank evaluation of autoimmune hemolytic anemia.

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Of course, not all hemolysis is mediated by the immune system. Some hemolysis is mediated by other conditions, such as erythrocyte membrane fragility or shearing effect. But, when the immune system does mediate hemolysis, it generally falls into one of three categories:

The first category is autoimmune hemolysis. This is when the immune system produces an antibody which binds to the patient's endogenous red blood cells. When these antibodies bind, they can cause either immediate destruction of red blood cells within the circulatory system itself (this is known as intravascular hemolysis) or can shorten the lifespan of red blood cells, usually due to increased clearance in the spleen (which is known as extravascular hemolysis).

The second category is alloimmune hemolysis. This is when the immune system produces an antibody as a result of an exposure to a non-self red blood cell antigen, for example, during a red blood cell transfusion or during a pregnancy. If the patient is then re-exposed to the non-self antigen, for example during a subsequent red blood cell transfusion, then the antibodies can mediate the destruction of the red blood cells. This is known as a hemolytic transfusion reaction. Another important type of alloimmune hemolysis is hemolytic disease of the fetus/newborn, or HDFN. In this entity, maternal

antibodies cross the placenta and mediate hemolysis of fetal red blood cells, sometimes resulting in fetal hydrops.

The third category is drug-induced hemolysis. This is really a quasi category, because some entities within this category behave more like autoimmune hemolytic anemia, while others behave more like alloimmune hemolytic anemia. However, this is when the immune system produces red blood cell antibodies after being exposed to a pharmaceutical agent (most commonly, an antibiotic). There are several different mechanisms, but the most common scenario is the so-called “drug dependent mechanism”, which require the offending pharmaceutical to be added to the test mechanism in order for the pathological antibody to be detected.

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In this brief talk, I will be focusing on the autoimmune hemolytic anemia category. The relevant entities within this category are: warm autoimmune hemolytic anemia (or WAIHA), cold agglutinin syndrome (or CAD), mixed IgG warm and IgM cold hemolytic anemia (or mixed), and rare entities, including IgM warm, IgG cold, and DAT negative hemolytic anemia.

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One extremely important concept is that these diagnoses cannot be made based on blood bank testing alone. The positive predictive value of a positive direct antibody test, or DAT, for immune hemolysis is believed to be less than 2%. Therefore, before any diagnoses are entertained, it is crucial for an initial workup and physical exam to be supportive, or at least partially supportive, of immune hemolysis.

Factors that are consistent with autoimmune hemolysis include: anemia, jaundice, dark urine, splenomegaly, derangement of hemolysis labs (such as the LDH test, the haptolgobin test, and the indirect bilirubin test), and evidence of red blood cell clearance on peripheral blood smear.

If the baseline lab studies and physical exam are at least partially supportive of a diagnosis of immune hemolytic anemia, the next step is to entertain other, more common diagnostic entities. Relevant questions include: is the patient bleeding? Is there evidence of mechanical – that is, non immune – destruction of red blood cells? Does the patient have a hemoglobinopathy or known red blood cell abnormality that could explain the destruction of the cells?

If, after considering these questions, immune hemolysis continues to be a reasonable possible diagnosis, then performing a DAT is a very reasonable option.

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Warm autoimmune hemolytic anemia is the most common type of immune hemolysis, but the overall incidence is still quite low. It is generally mediated by IgG class antibodies, which optimally react with the patient's own red blood cells at body temperature. While all ages and sexes can be affected, the most common clinical scenario is an older patient with an underlying hematological disorder, such as lymphoma. Fortunately, many of these patients do quite well with a combination of steroids and treatment of the underlying medical condition.

It is important to be aware that these patients generally do not die of anemia, per se. However, they are believed to be 5-10 times more likely to have a thromboembolic event compared to the average population. In addition, these patients sometimes have thrombocytopenia associated with their hemolytic anemia. This phenomenon is known as Evan's syndrome.

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Patients with WAIHA generally have a positive DAT for IgG, either alone or in combination with a positive DAT for complement. In some cases, the DAT is negative for IgG and is positive only for complement, although this may be a historical artifact based on less sensitive testing methodologies.

Elution studies in WAIHA typically reveal a panagglutinin – that is, an antibody which agglutinates all of the reagent red blood cells. In addition, the patient's antibody screen is often positive, with a panagglutinin identified during antibody ID. Sometimes, the antibody screen is negative, but the panaggultinin is identified in the eluate. This may represent a low level antibody that has not yet saturated the patient's endogenous red blood cell binding sites.

In these cases, it is important to exclude the possibility of an alloantibody in addition to the autoantibody. This can be approached by obtaining a detailed history (a man with no history of transfusion is much less likely to have an alloantibody than a woman with a history of pregnancy and previous red blood cell transfusion), along with adsorption studies, when applicable. Because these warm reacting autoantibodies can frustrate serologic investigations, some transfusion services obtain a

phenotype using molecular methodology, and then provide phenotype-matched blood to patients when an underlying alloantibody cannot be excluded.

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Cold agglutinin syndrome – or CAS – is significantly rarer than WAIHA. In addition, the symptoms are generally more severe. Indeed, the IgM antibodies that mediate CAS can result in intravascular hemolysis. It is important to emphasize that the detection of cold autoantibodies – for example, with non specific reactivity on antibody screen, or the incidental detection of agglutination on a peripheral smear – generally does not imply a diagnosis of CAS. Only patients with hemolysis as a result of these antibodies can be diagnosed with CAS.

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Patients with CAS have a DAT test that is positive for complement alone. If a 4°C titer is performed, it should be at least 64, and generally greater than 1000, to raise suspicion for CAS. Interestingly, the titer at 4°C seems to be less clinically important than the thermal amplitude of the antibody (that is, antibodies that react at near or at body temperature are more likely to mediate significant hemolysis than high concentrations of antibodies that react only in the cold).

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Mixed IgG warm and IgM cold hemolytic anemia – generally called mixed autoimmune hemolysis – is rarer than WAIHA or even CAS. In these cases, the DAT is positive for both IgG and complement, and cold agglutinins are detectable both at cold temperatures, and at body temperature. Severe cases of WAIHA are sometimes miscategorized and a mixed hemolysis, which is probably why some sources report steroid therapy to be effective treatment. Unfortunately, true cases of mixed hemolytic anemia are quite rare and are associated with a very dismal prognosis. There is no clear effective treatment with these cases, although some practitioners have had some limited success performing whole blood exchanges. With mixed autoimmune hemolysis, the most important consideration is bringing any underlying disease which may have triggered this event under control as rapidly as possible.

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So, we have now discussed the most common varieties of autoimmune hemolytic anemia: WAIHA, CAS, and mixed autoimmune hemolysis. Let's now briefly discuss the DAT pattern of reactivity for some less common diagnosis.

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For the sake of organization, the rarer varieties of autoimmune hemolytic anemia include: IgM warm hemolysis, IgG cold hemolysis, and DAT negative hemolysis.

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IgM warm hemolysis is rare, and I will only discuss it briefly. The DAT result in these cases would likely show reactivity only with complement reagents. In this situation, the IgM antibody is probably present, but just not detectable. Depending on the methodology used on the antibody screen, the result may be positive, due to carryover.

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You have probably heard about the clinical syndrome associated with IgG cold hemolysis – which is known as paroxysmal cold hemoglobinuria - but you may not realize the connection to the blood bank. This entity is rare, but when it is found, it is almost always in children with a recent viral infection. Presenting symptoms are generally consistent with hemolysis, and the Donath Landsteiner test – which sequentially incubates the patient's plasma with reagent RBCs at 4°C and then 37°C – should generally be performed to confirm the diagnosis. In these cases, the DAT is tricky. It is tempting to assume that the DAT would be positive for IgG, since the offending antibody is an IgG class antibody. However, classically the DAT is negative for IgG but positive for complement. This is probably due to the weak attachment of anti-P to the surface of the RBC, which allows it to be easily washed off during blood bank testing. However, the complement generally remains firmly attached.

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DAT Negative AIHA is really a diagnosis of exclusion. This diagnosis indicates that the clinical suspicion for immune hemolysis is high, but the DAT is negative. One important possibility to consider is that the DAT would be positive if additional reagents (such as anti-IgA) were available for clinical testing. Another possibility is that complement or IgG are present on the surface of the cell, but at concentrations that are just too low to be detected by commonly available testing systems. Some centers perform a "Super

Coombs" test to confirm this diagnosis; however, very few labs are able to perform this testing in house due to the difficulty of procuring appropriate positive and negative controls.

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This is the way that I think about the categorization of immune hemolytic anemia. Let the buyer beware! If there is clinical evidence or suspicion for hemolysis, then it is appropriate to perform a DAT, so long as we remember that the positive predictive value of a DAT for autoimmune hemolysis is very low.

If the IgG DAT is positive, then the most likely diagnosis is warm autoimmune hemolytic anemia. However, if the eluate is negative, then drug-induced immune hemolytic anemia should be considered. The reason that the eluate is negative in these rare cases is that the drug needs to be added to the assay in order to be detected.

If on the other hand, IgG DAT is negative, and the C3 DAT is positive, then additional testing is needed to determine if the diagnosis should be WAIHA or CAS. Factors that increase the likelihood of a CAS categorization include: room temperature agglutination and a 4°C titer >1000. The absence of room temperature agglutination makes WAIHA more likely than CAS. However, if room temperature agglutination is present, but the 4°C titer is <1000, then it may be that you are detecting cold agglutinins, but that their clinical significance is uncertain. Correlation with clinical details is strongly recommended in general, but especially in cases like this.

Finally, if both the IgG and the C3 DAT are negative, then the differential diagnosis should include DAT negative hemolysis, non-immune hemolysis, or, in areas such as the southeast United States where brown recluse spiders are present and the patient reports a recent insect bite, consideration should be given to a diagnosis of brown recluse spider bite mediated hemolysis (systemic loxoscelism).

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Immune hemolytic anemia is a medically important category of hemolytic anemia. Different categories of immune hemolysis have characteristic patterns of DAT reactivity. These patterns can overlap, making at times making the precise diagnosis a challenge.

It is important to repeatedly emphasize that hemolytic anemia should not be diagnosed based on blood bank testing alone. There should be other clinical and laboratory data to confirm the diagnosis – the blood bank should ideally only be determining how to best characterize the hemolytic process. Of equal importance: Immune hemolysis should not be excluded based on blood bank testing alone (e.g., "DAT negative" cases). Not every immunoglobulin type is tested for, so if the clinical suspicion is high enough, it is best not to exclude autoimmune hemolysis based on lab testing alone.

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Here are the references for this talk. Thank you for joining me on this Pearl of Laboratory Medicine on **"**Blood Bank Evaluation of Autoimmune Hemolytic Anemia."