rounds [hematology | chemistry]

Cold Agglutinin Syndrome

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Case Presentation

A CBC was performed on an 87-year-old woman with a diagnosis of deep vein thrombosis. The patient also had chronic lymphocytic leukemia. On the Cell Dyn 4000, all the results were invalid [T1]. The technologist checked the tube and there was grainy residue sticking to the sides of the tube. Suspecting cold agglutinins, he warmed the tube at 37°C for 20 minutes. He ran a CBC right away on the Cell Dyn 4000, but the results were once again invalid. However, on the Cell Dyn 3500, the differential results were valid but the Hgb and Hct results did not match (Hgb x 3 = ±3 Hct), as shown in T2. The Cell Dyn 3500 has a stronger lyse reagent, enabling it to produce a valid differential result.

To rule out problems with the draw, the phlebotomist was instructed to redraw the EDTA tube. An EDTA and a Sodium Citrate tube were drawn and a CBC was run, but the results remained the same, even after warming. The technologist made a slide that was extremely grainy, and when it was stained, it showed large clumps of red cells.

Invalid Results from Cell-Dyn 4000 T1

WBC	5.72*10e3/µL			
NEU	2.48*	%N	43.4*	
LYM	2.76*	%L	48.2*	
MONO	.267*	%M	4.67*	
EOS	.158*	%E	2.77*	
BASO	.050*	%B	.871*	
RBC	.311*	10e6/µL		
HGB	13.8*	g/dL		
HCT	2.84*	%		
MCV	91.4*	fL		
MCH	444.*	pg		
MCHC	486.*	g/dL		
RDW	29.0*	%		
RETC	----	10e3/µL	%R	----
IRF	----			
NRBC	0.00*	10e3/µL	NR/W	0.00*
PLT	277.*	10e3/µL		
MPV	9.21*	fL		

**Indicates invalid results*

Repeat Results of Cell-Dyn 4000 T3

WBC	8.64	10e3/µL		
NEU	3.38	%N	39.1	
LYM	4.39	%L	50.8	
MONO	.567	%M	6.56	
EOS	.288	%E	3.33	
BASO	.026	%B	.299	
RBC	3.38	10e6/µL		
HGB	12.6	g/dL		
HCT	32.7	%		
MCV	96.9	fL		
MCH	37.3	pg		
MCHC	38.5	g/dL		
RDW	12.5	%		
RETC	----	10e3/µL	%R	----
IRF	----			
NRBC	0.00	10e3/µL	NR/W	0.00*
PLT	216.	10e3/µL		
MPV	8.00	fL		

Valid Results from Cell-Dyn 3500 T2

WBC	8.10	K/µL	
NEU	3.37	41.6	%N
LYM	3.86	47.6	%L
MONO	.443	5.46	%M
EOS	.304	3.75	%E
BASO	.132	1.63	%B
RBC	1.42		M/µL
HGB	12.0		g/dL
HCT	14.8		%
MCV	104.		FL
MCH	84.6		pg
MCHC	81.0		g/dL
RDW	14.2		%
PLT	221.		K/µL
MPV	10.2		fL

The supervisor was notified of the problem, and he determined that the patient had very strong cold agglutinins. He suggested redrawing the patient and keeping the blood at 37°C until the specimen could be analyzed. Two technologists and the phlebotomist returned to the patient's room to redraw her. They brought a Styrofoam cup with water that had been warmed to approximately 40°C. The cup was covered with parafilm and a thermometer was placed in it to ensure that the temperature did not go below 37°C. It was then placed in 3 other Styrofoam cups to prevent heat from escaping. As soon as the phlebotomist drew the EDTA tube, she handed it to the first technologist, who at once placed it in the warm water and immediately transported the specimen to the laboratory. In the meantime, the phlebotomist drew another EDTA tube and handed it to the second technologist, who made slides right away.

The first specimen was run on the Cell Dyn 4000 immediately, and all the results were valid except for the MCHC, which was elevated [T3]. This alerted the technologist to review the slide for RBC morphology. The first specimen was also run in the Cell Dyn 3500, and although the differential results were valid, the Hgb and Hct did not match [T4]. There was only about a 15 second delay between running a CBC on the 2 instruments.

Autoimmune Hemolytic Anemia

Harmening describes autoimmune hemolytic anemia (AIHA) as representing "an abnormality within the immune system whereby the ability for self-recognition of an individual's own red cell antigens is lost. As a result, patients destroy their own red cells by producing autoantibodies, which bind to the patients' erythrocytes."[1] In some people, this is caused by a transient infectious disease and in others, the cause may be idiopathic.[2]

Autoimmune hemolytic anemia may be categorized into 2 types: the warm and cold types. The case described previously represents the cold type of AIHA. The cold type of AIHA can also be divided into 2 types, which are (1) Cold Agglutinin Syndrome and (2) Paroxysmal Cold Hemoglobinuria or PCH. T5 shows a list of differences between the 2 types:

The patient described previously has cold agglutinin syndrome. According to the Catholic Healthcare West Library, in addition to the causes listed in T5, cold agglutinins may also occur after influenza, atypical pneumonia, scleroderma, staphylococcemia, cytomegalovirus infection, hemolytic anemia, malaria, cirrhosis, congenital syphilis, peripheral vascular disease, pulmonary embolism, typanosomiasis, and tonsillitis.

Cold Agglutinin Syndrome

The cold agglutinin antibody is found to have anti-I or anti-i specificity, which is present in all adult RBCs. These autoantibodies are present to a lesser or greater degree in the serum of normal, healthy individuals. However, these autoagglutinins are not considered significant in normal, healthy individuals, as most of these cold autoantibodies are present in low concentration in the serum. They do not react at body temperature, are reactive optimally at lower temperatures, and are often too weak to even be detected in serologic

procedures. It is only significant in patients with the cold agglutinin syndrome.[1]

Mechanism of Cold Agglutinin Syndrome

The mechanism of cold agglutinins is as follows: when a person with this syndrome is exposed to the cold, the cold autoantibody is activated, which causes agglutination of RBCs and fixes complement as the RBCs flow through the capillaries of the skin. This results in autoagglutination and signs of acrocynosis (bluish tinge in extremities). Complement fixation may result in intravascular hemolysis. Patients usually display weakness, pallor, and weight loss, which are characteristic of chronic anemia. Physical findings such as hepatosplenomegaly are infrequent owing to mechanism of hemolysis. Other clinical features of cold hemagglutination diseases include jaundice and Raynaud's phenomenon (symptoms of cold intolerance, such as pain and a bluish tinge in the fingertips and toes, owing to vasospasm).[1]

In *Mycoplasma pneumoniae* infection, the organism responsible for these infections has an I-like antigen resulting in the production of anti-I by the body's immune system. Following the infection, the antibody is still present and since all red cells contain the I antigen, the antibody then becomes an autoantibody and starts reacting against the patient's own red cells. However in Epstein-Barr virus infection, the organism has an i-like antigen, thus resulting in the production of anti-i. This is a transient process that lasts only for a few weeks and seldom requires more than supportive care.[3]

Laboratory Findings

Often the first indication of the presence of cold agglutinins is the failure to obtain a meaningful RBC count and indices. The hemoglobin and hematocrit results do not match. The RBC count will be decreased due to doublet erythrocytes being counted as a single cell, thus resulting in a falsely high MCV. Hematocrit will also be lowered, as the volume of doublets are slightly less than 2 cells. The MCHC and MCH values will be increased due to decreased hematocrit and RBC count. Invalid red blood cell indices also can be due to lipemia, hemolysis, icterus, or hereditary spherocytosis. However, one can rule out lipemia, hemolysis, and icterus by visually inspecting the plasma or serum. Hereditary spherocytosis can be ruled out by evaluating the slide where many spherocytes should be visible.[5]

Repeated Results of Cell-Dyn 3500 T4

WBC	6.90	K/µL	
NEU	2.76	40.0	%N
LYM	3.67	53.2	%L
MONO	.254	3.69	%M
EOS	.186	2.69	%E
BASO	.028	.410	%B
RBC	1.77		M/µL
HGB	10.7		g/dL
HCT	17.8		%
MCV	101.		FL
MCH	60.3		pg
MCHC	59.9		g/dL
RDW	12.0		%
PLT	163.		K/µL
MPV	8.53		fL

Other laboratory findings include reticulocytosis, positive DAT, autoagglutination and/or rouleaux on peripheral blood smear, polychromasia, and mild to moderate anisocytosis and poikilocytosis. Agglutinates can also be visible in the specimen tube and can be pronounced as to give the

Paroxysmal Cold Hemoglobinuria versus Cold Agglutinin Syndrome T5

	PCH	Cold Agglutinin Syndrome
Patient Population	Children or young adults	Elderly or middle-aged
Pathogenesis	Following viral infection	Idiopathic/lymphoproliferative disorder/following *Mycoplasma* pneumoniae or Epstein-Barr virus infection
Clinical features	Hemoglobinuria: acute attacks upon exposure to cold (symptoms resolve in hours or days)	Acrocyanosis/autoagglutination of blood at room temperature
Severity of hemolysis	Acute and rapid	Chronic and rarely severe
Hemolysis	Intravascular	Extravascular/intravascular
Autoantibody	IgG (anti-P specificity) (biphasic hemolysin)	IgM (anti-I/i)(monophasic)
DAT	3+ (polyspecific Coombs' sera)/neg IgG/ 3-4+ C3 monospecific Coombs' sera	3+ (polyspecific Coombs' sera)/neg IgG/ 3-4+ C3 monospecific Coombs' sera
Thermal range	Moderate (<20×C)	High (up to 30 to 31°C)
Titer (4°C)	Moderate (<64)	High (>1000)
Donath-landsteiner test	Positive	Negative
Treatment	Supportive (disorder terminates when underlying illness resolves)	Avoid the cold

appearance of a large clot. Spherocytes may be present in the peripheral blood smear. The leukocyte and platelet counts are usually normal. Bilirubin is mildly elevated and rarely more than 3 mg/dL. The LDH levels may be increased, reflecting RBC destruction; and complement and haptoglobin can be low or absent. In brisk hemolysis, hemoglobinuria and hemoglobinemia manifest.[3]

Methods Testing Cold Agglutinins

Method 1. Rapid Screen for Cold Agglutinins:[4]

1. Place specimen in a water bath at 37°C as soon as it arrives in the laboratory. Maintain at 37°C until serum and cells are separated.
2. Separate the serum from the clot when the clot has retracted completely.
3. Using a Pasteur pipette, transfer the serum into a labeled tube and centrifuge at 400 rpm for 2 minutes.
4. Prepare a 5% suspension of the patient's cells.
5. Set up 2 test tubes in a test tube rack.
6. Place 2 drops of the patient's serum in each tube. To each tube add consecutively:
   a. 1 drop of patient's RBCs.
   b. 1 drop of group O cord RBCs.
7. Incubate the serum-cell mixture at 20°C for 1 hour.
8. Read and record results.
9. Incubate the same tubes in a water bath at 15°C for 1 hour.
10. Read and record results.

Interpretation:

If agglutination is observed at 15°C and 20°C, proceed with the titration techniques as described in Method 2. If agglutination is observed at 15°C but disappears as the tube is warmed to 20°C or higher, record the result as negative.

Method 2. Titration of Cold Agglutinins:[4]

1. Set out 10 tubes in a test tube rack.
2. Place 1.5 mL of diluent (physiologic saline) in tube 1 and 1.0 mL of diluent (physiologic saline) in tubes 2 through 10.
3. Add 0.5 mL of the patient's serum to tube 1, mix and transfer 1.0 mL to tube 2, mix and transfer 1.0 mL to tube 3, and so on until tube 9. Tube 10 will serve as a cell control. By this method, dilutions will be 1:4 to 1:1,024.
4. Add 0.1 mL of the patient's own RBCs (2% suspension) to each tube.
5. Mix the contents by vigorously shaking the test tube rack and place racks at 4°C overnight.
6. Remove the tubes from the refrigerator and read immediately.

Interpretation:

The titer of cold agglutinins is read as the reciprocal of the highest dilution exhibiting any agglutination (1+ agglutination is usually taken as the weakest when read macroscopically). After reading, place the tubes at 37°C for 2 hours and reread. If the titer was due to cold agglutinins, the erythrocytes will be dispersed and no agglutination will be seen.

Dealing with Cold Agglutinins

In cases where the patient does not have a very strong cold agglutinin, the specimen can be placed in a 37°C incubator for at least 5 minutes and then run right away. However, in cases like the one described here, the blood needs to be maintained at 37°C until it can be tested. Some suggested methods for keeping the specimen at 37°C are by using a portable water-bath as described in this case or by using a heel-warmer. The tube can also be prewarmed. Another recommendation is to draw the patient with a butterfly while having the tube sit in a warm cup of water. If possible, the phlebotomist should draw the patient near the instrument so that the specimen can be run immediately. For patients with really strong cold agglutinins, the reagents on the instruments may be warmed if the instrument allows it.

Treatment

The most common and easiest treatment for cold agglutinin syndrome is to avoid the cold, keep warm, or move to a warmer climate. In more severe cases, plasma exchange may be performed. Other methods of treatments include prescribing corticosteroids for patients whose RBCs have been highly sensitized with C3. Alkylating drug chlorambucil has some favorable results as well. If blood transfusion is required, blood should be transfused warm.[5]

Conclusion

Although cases of mild cold agglutinins are routinely met in the laboratory, strong cold agglutinins are not. When met, technologists who are not familiar with severe cases as described in this article may not know what to do. This paper was written with the intention of alerting technologists to the presence of strong cold agglutinins and its effects on laboratory tests. Knowledge of this phenomenon can help prevent too much time being wasted on solving the problem as well as sparing the patient the ordeal of unnecessary repeat draws.

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Additional Information

Synonyms, Key Words, and Related Terms: cold agglutinin hemolytic anemia, cold agglutinin disease (CAD), acrocyanosis, cold-induced immune hemolytic anemia, mixed autoimmune hemolysis

Web site description at:
http://www.emedicine.com/med/topic408.htm. Accessed on April 30, 2002. Authored by Rajalaxmi McKenna, MD, FACP and Harry L. Messmore, Jr., MD. Site contains treatment regimens for physicians.

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