COLD AGGLUTININ DISEASE - HOW TUBE TECHNIQUE HELPED US IN RESOLVING DISCREPANCY

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Abstract:
Introduction - Cold agglutinin disease is associated with circulating antibodies that react preferentially in cold temperatures. Patients present with hemolytic anemia, acrocyanosis and hemoglobinuria. While column agglutination technology is very helpful in warm red cell antibodies, it has its own disadvantages in resolving cold antibodies. Case report - A 54 year old female was admitted in the intensive care unit with complaints of breathlessness, weakness and extreme fatiguability. Her Hb was 8.5 gm. There was spontaneous agglutination of collected blood samples and all laboratory parameters tested were spurious so, she was suspected to have immune haemolytic anemia. The sample was then warmed to 370 C for 30 minutes which cleared all agglutination. The 2-5 cell suspension was maintained at 370 C. Thermal amplitude of the antibody showed the titre to be 1024 at 40 C. When crossmatching was performed with gel column, red cell units were incompatible (2 reactions) but crossmatching done with tube technique maintaining samples at 370 C found two red cell units to be compatible. The patient was advised to be shifted out of the air conditioned ICU and kept in warm environment. She was transfused with two units of crossmatch compatible packed red blood cells, slowly with the help of blood warmer. Conclusion - Cold agglutinin disease poses difficulty in evaluating patients as their blood samples agglutinate spontaneously at room temperature. The column agglutination technology is not very helpful in evaluating cold agglutinins, especially when the agglutinins have high thermal amplitude as the temperature is not maintained at 370 C while centrifuging. Tube technique, is very simple and the prime modality in resolving cold agglutinins where the CAT fails.

Keyword: cold agglutinin, tube technique, column agglutination technology

Introduction:
Cold agglutinin disease is associated with circulating antibodies that react preferentially in cold temperatures. The acute form of this disease is often secondary to infections like Mycoplasma pneumoniae, Ebstein Barr virus, Cytomegalo virus, Malaria, etc. while chronic form is often seen in elderly associated with lymphoma, leukemia or Waldenstrom macroglobulinemia. 1, 2. Patients present with hemolytic anemia, acrocyanosis and hemoglobinuria, while laboratory data show low hemoglobin, raised reticulocyte count and raised bilirubin, reduced haptoglobin, raised LDH levels and changes in red cell morphology indicating hemolysis. Investigations carried out at blood bank in cases of hemolytic anemia help to determine immune basis and, if so, the type of immune hemolytic anemia. 1 Column agglutination technology (CAT) has become the easy and less time consuming technique for investigation in immune hemolytic anemia. While CAT is very helpful in warm red cell antibodies, it has its own disadvantages in resolving cold antibodies. In these situations, the age old test tube technique, which is the gold standard, helps to resolve discrepancies and identify cold red cell antibodies more effectively than CAT.

Case report:
A 54 year old female was admitted in the intensive care unit of a hospital with complaints of breathlessness, weakness and extreme fatiguability for one week. She gave a history of cough and rashes for 2 days three weeks back, for which she had consulted a hospital and was given supportive treatment and hematinics. Her rashes subsided, but she developed easy fatiguability. She had visited another physician who ordered blood investigations. Her Hb was 8.5 gm% and so was referred to higher centre for evaluation. Evaluation was difficult at the tertiary hospital as there was spontaneous agglutination of collected blood samples. As all laboratory parameters tested were spurious, she was suspected to have immune haemolytic anemia and sample was sent to our blood bank for work up. The EDTA sample received was strongly agglutinated at room temperature and blood grouping could not be carried out. So the sample was warmed to 370 C for 30 minutes which cleared all agglutination (confirmed microscopically). The sample was washed in test tubes thrice with warmed saline (370 C), with the help of warmed centrifuge tubes. The 2-5% cell suspension was maintained at 370 C with the help of water bath. Blood grouping and Rh typing was performed using warmed test tubes, warmed reagents and found to be A positive. Autocontrol was negative when sample was maintained at 370 C. So it was confirmed as a cold agglutinin. Thermal amplitude of the antibody was found by incubating the patient’s plasma with O pooled cells and titre was worked out. The titre was 1024 at 40 C, 128 at 220 C, 8 at 300 C and 0 at 370 C. Crossmatching was performed by maintaining both donor cells suspension and patient’s plasma at 370 C (in water bath) using prewarmed centrifuge test tubes and two donor units were found to be compatible (confirmed microscopically). When crossmatching was performed with gel column, the red cell
units were incompatible (2+ reactions). This incompatible result was deduced to be due to the automated centrifuge used in CAT technology, which centrifuges at room temperature for 10 minutes, which lowered the temperature of the sample (the cold antibody had high thermal amplitude) and had resulted in agglutination. The patient was advised to be shifted out of the air conditioned ICU and kept in warm environment. She was transfused with two units of crossmatch compatible packed red blood cells slowly, with the help of a blood warmer. She did not develop any reactions and improved significantly. Meanwhile, a short course of steroids (Inj. Methyl prednisone) helped her tide over the hemolytic crisis.

Discussion: A cold agglutinin is clinically significant when the antibody has a titre of > 1000 or with high thermal amplitude. In this patient, we found a titre of 1024 at 40°C with high thermal amplitude (300°C). The specificity of the cold agglutinin was not further evaluated. Infectious etiology was suspected, as the patient had cough, fever and rashes over body two weeks before the onset of anemia. But antibody tests (IgM, IgG) to find out infectious agent were not performed. The cold agglutinin is usually IgM antibody which binds complement to red blood cells in colder peripheral circulation. When the red cells reach warmer temperatures (370°C), the IgM antibody dissociates, but the complement system is activated causing hemolysis. The disadvantage of the column agglutination technology was that even though the serum and red cells are incubated at 370°C, the temperature is not maintained while centrifuging (which is done at room temperature). This caused the 1+ to 2+ agglutination reaction at the end of ten minutes centrifuge. The tube technique, which is the gold standard technique, was highly helpful in this cold agglutinin with high thermal amplitude, by allowing the samples to be maintained at 370°C throughout the procedure and also by the short centrifuge period.

Conclusion: Cold agglutinin disease poses difficulty in evaluating patients as their blood samples agglutinate spontaneously at room temperature. The column agglutination technology, which is very useful in warm antibodies, is not very helpful in evaluating cold agglutinins especially when the agglutinins have high thermal amplitude. Tube technique, is very simple and the prime modality in resolving cold agglutinins where the CAT fails.

References: