Case Report

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Direct antibody test negative autoimmune hemolytic anemia with pulmonary tuberculosis: A diagnostic challenge

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Abstract:

Tuberculosis (TB) has varied manifestations, but autoimmune hemolytic anemia (AIHA) due to TB is rare. Direct antibody test (DAT) or Coombs negative AIHA is also rare. We report a case of a 14-year-old boy who presented with hemolytic anemia and pneumonia. The Coombs test was repeatedly negative. After ruling out the possible infectious and noninfectious causes by extensive investigations, he was diagnosed as DAT-negative AIHA by monospecific antibody test with 4°C low ionic strength saline washes and column agglutination method which revealed the presence of IgG-2+ antibodies. Bronchoalveolar lavage fluid for acid-fast bacilli and gene Xpert was also positive. It is important to recognize TB as a cause of AIHA in South Asian countries where its incidence is high.

Keywords:

Autoimmune hemolytic anemia, Coombs negative, pediatric, tuberculosis

Introduction

Tuberculosis (TB) has varied manifestations, but autoimmune hemolytic anemia (AIHA) due to TB is rare. Direct antibody test (DAT) or Coombs negative AIHA usually due to red blood cell (RBC) bound IgG below the threshold of detection by standard methods is also rare and may pose a diagnostic challenge as in our case.

Case Report

A 14-year-old boy presented with a history of yellowish discoloration of the eyes for 15 months, progressive pallor, and low grade, intermittent fever for 1 year. He also had a dry cough for 2 months and breathing difficulty for 15 days. He was admitted for similar complaints 4 months back to another hospital, where he received three units of blood transfusion and antibiotics for 10 days. In between, the child was asymptomatic except for persistent mild jaundice. He was apparently growing well but was unimmunized. There was no history of blood transfusion, jaundice, and abdominal distension before 15 months. There was a history of TB in two family members in the past 2 years. There was no history of jaundice or cholelithiasis or blood transfusion in family. He belonged to the upper lower class with overcrowding in house.

At admission, the patient was febrile with severe respiratory distress and saturation of 62%. There were severe pallor and icterus but no lymphadenopathy or petechiae. Systemic examination revealed bilateral coarse crepitations and significant

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splenohepatomegaly (spleen - 7 cm, liver - 4 cm >costal margin). He was managed in the intensive care unit with noninvasive ventilation, blood transfusion, and antibiotics. Initial investigations showed hemoglobin of 4.2 g%, total leukocytes count – 13,000/mm^3 with polymorphs - 80%, serum bilirubin - 7.9 mg% with indirect bilirubin - 7.6 mg%, corrected reticulocyte count was 3%, and lactate dehydrogenase levels were raised (898 u/L). Peripheral smear was also suggestive of hemolytic anemia.

Investigations for immune and nonimmune causes for hemolytic anemia were planned. Direct antibody or Coombs test and indirect Coombs test were negative on three different occasions. G6PD level was also normal twice. Sickling test, osmotic fragility, flowcytometry for paroxysmal nocturnal hemoglobinuria, hemoglobin electrophoresis, anti-nuclear antibody (ANA), perinuclear form of anti-neutrophil cytoplasmic antibody (ANCA), cytoplasmic-ANCA, and serum ceruloplasmin were also normal. Work up for infectious diseases such as *Mycoplasma*, Epstein–Barr Virus, typhoid fever, Kala Azar, rickettsia, HIV, and hepatitis B and C, GeneXpert for M. tuberculosis of gastric aspirate was also negative.. Bone marrow aspiration was noncontributory.

Pneumonia was not resolving even after 15 days of good antibiotic cover, supportive care, and the patient required repeated transfusion for ongoing hemolysis. Hence, bronchoscopy and special investigations for AIHA were planned. Monospecific antibody test with 4°C low ionic strength saline washes (LISS) by column agglutination method was performed which revealed the presence of IgG-2+ antibodies. Bronchoalveolar lavage fluid for acid fast bacilli staining and gene Xpert were both positive. A diagnosis of Coombs/DAT negative AIHA with pulmonary TB was thus established. The patient was treated with antitubercular treatment (ATT). There was significant improvement within 7–10 days, child was afebrile, appetite improved, and there was no further fall in hemoglobin with no transfusion requirement later. On follow-up at 5 months, the hemoglobin level was maintained between 12 and 13 g% and there was weight gain of 6 kg. Moreover, there is no evidence of hemolysis on peripheral smear on follow-up.

Discussion

Secondary AIHA is due to molecular mimicry of foreign antigens that cross-react with RBC self-antigens usually Rh proteins.[1] Several infectious and non-infectious causes have been attributed which may cause warm or cold type of AIHA. Mycobacterium TB may cause an immune response leading to the production of IgG, IgM, or both and causing warm, cold, or mixed type of AIHA.[2] Coombs test or DAT which can detect RBC-bound IgG and complement (C3), is still an essential assay for the diagnosis of AIHA. However, 5%–10% of patients with AIHA have negative DAT findings and are labeled as "DAT negative AIHA."[3] The principal reasons for DAT-negative AIHA are: (a) RBC bound IgG may be below the threshold of detection by standard methods (<260–500 molecules/cell), (b) low affinity of IgG, or (c) RBC may be bound to IgA or rarely IgM antibody. This can be diagnosed with special tests such as follows:[4,5]

1. Column agglutination test: A column agglutination method-DAT (CM-DAT) that requires no washing procedure can detect low-affinity autoantibodies[6]
2. Concentrated eluate assay: Increased ratio of antibody to antigen[6,7]
3. Monospecific DAT: Antihuman globulins used in DAT can be poly-specific, where they will bind to IgG and certain complement components while in monospecific DAT, they bind only IgG or a specific complement component[8]
4. LISS: Low-affinity IgG antibodies may be removed from the red cell surface during preparative washing of the cells at 37°C or at room temperature. Cold washing with isotonic saline at 0°C–4°C or with low ionic strength saline can prevent the loss of IgG from the red cell surface, thereby retaining a positive reaction to the commercial DAT reagent[4]
5. Flow cytometry: Flow cytometry can be calibrated such that it can detect fluorescent-labeled anti-human IgG on red cells at a sensitivity greater than that of the commercial DAT reagent to detect false-negative antiglobulin test.[4]

TB affects all systems but hematological manifestations of TB are uncommon except anemia. AIHA is an extremely rare occurrence in TB.[9] A total of 21 cases (including adults) that have proven association of TB with AIHA have been reported so far in the English literature. Most of these are from India and only three cases are reported in children <18 years.[10] All cases were DAT-positive AIHA. About 46.7% were classified as warm AIHA, 40% as cold AIHA, and 13.3% as mixed type of AIHA.[10] Treatment with ATT and steroids may be required in few cases, especially in disseminated TB, cold, or mixed AIHA. Monoclonal antibodies (Rituximab) or splenectomy may be required rarely.[2,10] Bakhshi *et al*. reported an 8-year-old female with disseminated TB and mixed AIHA treated with ATT and steroids.[11] Gupta and Bhatia also reported an 8-year-old male child with abdominal TB and warm AIHA treated with ATT and steroids.[12] In 2008, Khemiri *et al*. reported an 11-year-old female with pulmonary TB and warm AIHA with immune thrombocytopenia who was successfully treated with ATT alone.[13]

Our case was unique and challenging because the coombs test was negative multiple times. Furthermore,

the gastric aspirate for GeneXpert was negative. Hence, a high index of suspicion was necessary for the diagnosis after an extensive workup which ruled out almost all infectious and noninfectious causes for hemolytic anemia. Pulmonary TB was confirmed after broncho-alveolar lavage fluid tested positive for AFB and Gene -Xpert then we gave ATT without steroids to which the child responded and repeat specific tests came negative.

Conclusion

Both DAT-negative AIHA and TB induced AIHA are extremely rare. However, the incidence of TB is high in South-Asian countries and hence it is important to recognize TB as a cause of AIHA. Fiberoptic bronchoscopy should be instituted in cases with strong suspicion of TB where it can yield good results.

Informed consent

Case report consent was obtained with the patient attendant before submitting the article.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the legal guardian has given his consent for images and other clinical information to be reported in the journal. The guardian understands that names and initials will not be published and due efforts will be made to conceal patient identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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