Autoimmune Hemolytic Anemia: Mixed Type—A Case Report

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Abstract Mixed autoimmune haemolytic anemia (AIHA) is defined by the presence of both warm and cold auto antibodies. Diagnosis is based on detection of autoantibodies by monospecific direct antiglobulin test showing a pattern of IgG and complement C3d and presence of cold agglutinins. We report a rare case of primary mixed AIHA in a 12 year old girl who responded to corticosteroids.

Keywords Autoimmune haemolytic anemia (AIHA) · Direct antiglobulin test (DAT) · Auto agglutination · Corticosteroids

Introduction

Autoimmune haemolytic anemia (AIHA) consists of a group of acquired haemolytic anemias which result from the development of auto antibodies directed against antigens on the surface of patient's own red blood cells [1]. Based on temperatures at which auto antibodies react with red blood cells, AIHA is classified into warm and cold antibody types. Warm antibody AIHA occurs predominantly in children aged 2–12 years. They belong to IgG class, react at temperatures $\geq 37^{\circ}\text{C}$, do not require complement for activity and do not produce agglutination in

vitro. On the other hand cold antibody AIHA occurs less commonly in children. Antibodies are of IgM class which react at temperatures $< 37^{\circ}\text{C}$, require complement for activity and produce spontaneous agglutination of red blood cells in vitro [2]. Mixed warm and cold antibody AIHA is the presence of both warm and cold auto antibodies [3].

Autoimmune haemolytic anemia is an uncommon cause of haemolytic anemia in children with an incidence of approximately 0.2 per 100,000 in 11–20 years age group. Occurrence of mixed type of AIHA is rare with $< 5\%$ of all cases of AIHA reported and in children they are extremely rare [4, 5]. We report one such rare case of mixed warm and cold type of AIHA in a 12 year old girl with review of literature.

Case Report

A 12 year old girl presented to the Emergency Department with fever, headache, abdominal pain, vomiting and yellowish discoloration of eyes of 5 days duration. Physical examination revealed marked pallor, fever, tachycardia, tachypnoea and icterus. There was no lymphadenopathy, edema, rash, petechiae or bruises. Cardio vascular examination revealed a 3/6 systolic murmur along the left sternal border. A non tender soft hepatomegaly with a span of 14 cm and a soft spleen 3 cm below the left costal margin was noted. Lung fields were clear and neurological examination was normal. Fundoscopy revealed disc edema, Roth spots, fusiform dilatation of veins with extravasation and arteriolar haemorrhages.

Automated cell counter of peripheral blood showed a normal total leucocyte count (TLC) with normal differentiation and a normal platelet count. Evaluation of blood

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parameters revealed features of severe anemia [haemoglobin (Hb)—3 g/dl] with evidence of macrocytosis (MCV—128.1 fl) and increase in MCH (50.9 pg) and MCHC (39.7 g/dl) also. Agglutination of red blood cells was noted on peripheral smear examination with separation on warming the slides (Figs. 1, 2). Smear showed anisopoikilocytosis with predominant macrocytes, hypochromia and nucleated red blood cells. Malarial parasite was absent. Reticulocyte count was 10%. Direct anti globulin test (DAT) was strongly positive. Liver function tests showed a total serum bilirubin (TSB) of 4.5 mg/dl with an indirect fraction of 3.2 mg/dl and normal enzyme levels. Urine analysis was normal.

Clinical presentation and laboratory parameters were consistent with hemolysis and a working diagnosis of AIHA was made.

Patient's history further revealed that she was hospitalised 14 months ago for similar complaints following fever. Medical records (discharge summary from previous hospital) obtained at a later date revealed a diagnosis of AIHA for which the patient had received 2 units of packed red cells and corticosteroids (prednisolone). The patient had been discharged after 2 weeks with advice to continue steroids but she had discontinued the same after 2 weeks and had failed to go for follow up. She had been asymptomatic and was not on any medication till she presented to us.

Severe anemia necessitated transfusion with packed red cells. Difficulty was encountered during grouping and cross matching due to excessive agglutination of red blood cells. However group specific packed red cells which were 'least incompatible' was transfused. Auto agglutination of red blood cells at room temperature with reversal on warming the slide and a strongly positive DAT with evidence of hemolysis prompted us to revise our diagnosis as AIHA

Fig. 1 Autoagglutination of red blood cells at room temperature

Fig. 2 Separation of red blood cells on warming the slide

due to cold auto antibodies. To confirm our diagnosis; further screening was done with mono specific DAT, Indirect antiglobulin test (IAT) and antibody screening. Mono specific DAT showed both anti IgG and anti C3d antibodies while IAT was positive at 4°C and negative at 22 and 39°C. Antibody screening performed utilising a three cell panel of asia specific reagent red cells coated with Rh, Kell, Duffy, Kidd, Lewis, P, MNS, Lutheran and Xg antigens using gel card by microtyping technique showed panpositivity to all cell panels. The above findings indicated AIHA of mixed warm–cold type.

Further investigations were done to identify the etiology. Serum glucose levels and thyroid function tests were normal. Bone marrow aspiration showed reactive erythroid hyperplasia and there were no abnormal cells. The patient also had severe headache which in association with roth spots prompted us to investigate for other causes of infective etiology. CT scan of brain and ECHO were all normal. Blood and urine cultures were also negative. Screening for tuberculosis in the form of Mantoux test, chest radiograph and sputum for acid fast bacilli were negative. Serology for Human immunodeficiency virus, Syphilis, Epstein Barr virus, Cytomegalovirus, Hepatitis A, B, C viruses and Mycoplasma pneumonia were negative. Rheumatoid factors, anti nuclear antibody and lupus cells were negative. Hence a final diagnosis of AIHA of mixed warm and cold type of idiopathic nature was made.

The patient was started on intravenous ceftriaxone in view of fever and given 2 units of packed red cells to improve her Hb status. With the diagnosis of AIHA confirmed by DAT, patient was started on prednisolone (2 mg/kg/day) in divided doses. She developed pancytopenia on the 10th day of admission which improved spontaneously in the next 24 h. Subsequently, the patient had an uneventful course with regression of hepatosplenomegaly and maintenance of Hb levels at 11.8 g/dl. After 4 weeks of steroids, they were gradually tapered to a

maintenance dose of 0.5 mg/kg/day on alternate days. On follow up, her Hb was maintained at 12.4 g/dl with disappearance of roth spots on fundoscopy. Her polyspecific DAT continued to be positive while monospecific DAT was strongly positive for anti IgG and negative for anti C3d. Auto agglutination of red blood cells was also absent. The patient continued to be in remission 6 months later with DAT being negative. Hence it was concluded that the child had AIHA of mixed type with exacerbation following fever presumably of viral origin. Thereafter she has been followed up for 1 year and is still in remission on alternate day steroids.

Discussion

In children, haemolytic anemia is usually caused by intrinsic defects in red blood cells as seen typically in membrane defects, red cell enzyme deficiencies or haemoglobin abnormalities and most of them are inherited in nature. Hemolytic anemias also result due to factors extrinsic to red blood cells with most of the causes being acquired [1]. AIHA is one such group of acquired haemolytic anemias which results from the development of auto antibodies directed against antigens on the surface of patient's own red blood cells. Majority of the cases are mediated by warm reactive auto antibodies while AIHA due to cold reactive antibodies are less common [2]. Mixed type of AIHA is rare in the pediatric age group. Review of literature revealed very few cases of mixed type of AIHA [3, 6–9].

Diagnosis of AIHA is based on evidence of haemolytic anemia consisting of anemia, jaundice, splenomegaly, reticulocytosis, raised serum bilirubin and a positive DAT [1]. Once AIHA has been identified, differentiation between warm and cold antibodies can be done by monospecific DAT which also identifies responsible mechanisms. If the reaction is positive with anti IgG and negative with anti C3d, it is usually due to warm antibodies which are common in idiopathic or drug associated AIHA. If the reaction is positive with both anti IgG and anti C3d, it also indicates warm autoantibodies and is more common in patients with systemic lupus erythematosus (SLE) and idiopathic AIHA. In cold agglutinin disease (CAD), the reaction is positive with anti C3d but negative with anti IgG [10]. There is also presence of agglutination of red cells at temperatures <37°C in cold type of AIHA [2]. In the present case, initially a misdiagnosis of cold antibody AIHA was made based on positive polyspecific DAT, red cells agglutination at room temperature which reversed on warming the slide and presence of cold agglutinins at 4°C. However, monospecific DAT done subsequently was positive for anti IgG and anti C3d indicating warm

antibodies. So we revised our diagnosis as a mixed type of AIHA thereby emphasising the importance of monospecific DAT. On subsequent follow up, cold agglutinins had disappeared and monospecific DAT was strongly positive for anti IgG only indicating warm auto antibodies. Previous medical records did not mention auto agglutination of red cells nor was monospecific DAT done. Hence, the patient probably had a warm type of AIHA during the previous episode.

Autoimmune haemolytic anemia is known to be associated with infection, malignancy or other autoimmune diseases but in most cases it is idiopathic [1, 2]. Cause of AIHA in children remained obscure in majority of cases labelling them as idiopathic or primary [8, 11]. Associated medical conditions linked to AIHA such as viral infections, SLE, Mycoplasma pneumonia, immunisation, tuberculosis, diabetes mellitus, Hodgkin's lymphoma, auto immune hepatitis, sepsis with bacterial endocarditis due to Staphylococcus aureus, Guillain-Barré syndrome and Langerhan cell histiocytosis have been reported [3, 6–9, 11, 12]. Patients with AIHA exhibit normal granulocyte and platelet counts [1, 2]. Combination of AIHA with immune thrombocytopenia (Evans syndrome) has been described [6, 11, 12] but in the present case, the patient's platelet count was normal initially and there was no bleeding manifestation throughout her stay in hospital. During the course of treatment, platelet and leucocyte counts dropped precipitously for a transient period along with a fall in Hb. The cause for this transient pancytopenia was presumed to be due to a viral infection as the patient was highly febrile during that period. The link between AIHA and SLE is well established in medical literature. The initial manifestation may be AIHA, which may precede by months or years the other clinical manifestations of SLE [13] necessitating screening of all patients with mixed AIHA for evidence of SLE. We did not detect any evidence of SLE in our patient and she is being followed up for the development of any signs of the same.

Additional findings of roth spots and retinal haemorrhages noted in the present case prompted us to investigate for infective endocarditis (IE) which was negative. Roth spots are found in a variety of medical conditions other than IE and profound anemia is one of them. Ischemic insults to capillary endothelium of any etiology associated with elevated venous pressures can cause roth spots and retinal haemorrhages and as such is a non specific sign [14]. One case of AIHA associated with IE has been published, however roth spots have not been reported [8].

Corticosteroid therapy is the mainstay of treatment in warm AIHA and is less effective in CAD. Transfusions are of transient benefit but may be required initially because of severity of anemia. Transfusion of red cells in AIHA can be complicated because of cross matching problems and rapid

in vivo destruction of transfused cells due to the presence of auto antibodies [12, 15]. We encountered problems during blood grouping owing to clumping of red cells which was resolved by warming. Cross matching was also troublesome probably due to the presence of pan agglutinins and 'least incompatible' B +ve red cells were transfused. However there were no post transfusion hemolytic episodes. Immunosuppressive agents including monoclonal anti -CD 20 (Rituximab) may prove useful in CAD and refractory cases of warm AIHA. Splenectomy is of benefit in refractory cases of warm AIHA but is not useful in CAD [2, 16]. Patients with mixed type of AIHA show a dramatic response to steroid therapy and frequently require few or no transfusions [17]. Our patient received only two transfusions and responded well to corticosteroids.

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