Blood group discrepancy in mixed-type autoimmune hemolytic anemia in a pediatric patient

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Abstract:
Autoimmune hemolytic anemia (AIHA) is characterized by the presence of antibodies directed against self-antigens on red blood cells (RBCs) leading to progressive RBC destruction along with reduced red cell survival. Mixed-type AIHA is characterized by the presence of both warm and cold-autoantibodies. These autoantibodies may cause blood-group discrepancy or cross-match incompatibility leading to delay in arranging suitable blood unit for transfusion. The detection of autoantibodies by monospecific-direct antiglobulin test showing positive reaction on immunoglobulin G and C3d and presence of cold-agglutinins leads to the diagnosis. We report a rare case of mixed AIHA in a 15 years female showing severe anemia, blood group discrepancy, and cross-match incompatibility. She received transfusion of least incompatible packed RBCs without any untoward effect.

Keywords:
Autoantibodies, C3d, direct antiglobulin test, discrepancy, mixed autoimmune hemolytic anemia

Introduction
Blood-grouping involves both cell, i.e., forward-grouping and serum, i.e., reverse-grouping. Both the results should be in agreement with each other. Whenever there is a disagreement between forward and reverse-grouping, it is implied as a blood-group discrepancy. Blood-group discrepancies must be resolved prior to reporting a patient/donor blood-group and releasing a blood component for transfusion.[1]

Autoimmune hemolytic anemia (AIHA) is group of hemolytic anemia resulting due to autoantibodies directed against antigens on the surface of patient’s own red cells.[2] Depending on thermal amplitude of autoantibodies, it can be classified as warm and cold-type. Warm-antibodies are immunoglobulin G (IgG)-class, reacting at 37°C, do not require complement for activity or cause in vitro agglutination; whereas cold-antibodies are IgM-class, reacting at 0°C–4°C, requiring complement for activity and can produce spontaneous in vitro agglutination of red cells.[3] Mixed-type AIHA is characterized by the presence of both warm and cold-autoantibodies.[4]

Das et al. showed median age of mixed-type AIHA to be 37 years.[5] AIHA is uncommon in children with an incidence of about 0.2/100,000 between 11 and 20 years. Mixed-type AIHA is seen in <5% cases and rarely in children.[6,7] We report a rare case of mixed-AIHA in a 15 years female.

Case Report
A 15-year-old female came to the pediatric outpatient department with complaints of progressive fatigue, weakness, and...
breathlessness since 2 months. On general examination, there was severe pallor, rash over nose, tachycardia, and tachypnea. Per abdomen examination showed mild splenomegaly. She had no history of blood transfusion and did not have acrocyanosis or lymphadenopathy. She was admitted and investigated. Her hemoglobin was 4.2 g% with total red blood cell (RBC) count of 0.48 million/μl. Her total leukocyte count was 6700/μl and platelet count was 1.5 lac/μl. Peripheral smear showed agglutination of red cells, macrocytosis with anisopoikilocytosis and basophilic stippling. Malaria parasite was absent. Reticulocyte count was increased. Erythrocyte sedimentation rate was markedly increased (170 mm). Bone marrow aspiration showed erythroid hyperplasia. Direct antiglobulin test (DAT) was strongly positive. Indirect bilirubin was mildly increased (2.4 mg/dl) with markedly increased serum lactate dehydrogenase (1191 U/L). Urinalysis was normal. We received patient’s sample for blood grouping and cross-matching. Blood grouping performed by conventional tube technique showed forward and reverse discrepancy. Forward grouping (at room temperature) showed 4+ agglutination with anti-A, anti-B, anti-AB and anti-D; reverse grouping with in-house prepared pooled cells showed 4+ agglutination with A-cells, and 2+ agglutination with B-cells, O-cells, and autocontrol. Repeat blood-grouping was performed after prewarming at 37°C and washing with warm saline. Forward and reverse grouping performed at 4°C, 22°C and 37°C showed discrepant result [Table 1]. Repeat testing by conventional tube technique and gel-card technique with fresh sample showed the same results. Poly-specific DAT (IgG + C3d) was positive (3+). Antibody screening and identification performed by 3 and 11 cell-panel by gel card technique showed pan-positivity (2+). All these findings suggested the diagnosis of AIHA-mixed type.

Her sample was cross-matched by gel-card technique with two B-positive and two O-positive units but was incompatible (4°C −4+, 22°C −4+, 37°C −2+). She was issued and transfused least incompatible B-positive packed RBC (PRBC) unit without any transfusion reaction. Steroid therapy was advised and started. Her blood sample was sent again after 2 days of steroid therapy. Her blood group was confirmed as B-positive and cross-match was also compatible [Table 2]. She responded to steroid and hematinsics therapy. Her clinical condition improved and was discharged with an advice to follow-up. On further work-up ANA was positive, but dsDNA was negative. Anti-smith antibodies, anti-RNP antibodies were positive and diagnosis of overlap syndrome of mixed connective tissue disorder was considered as she had polyarthralgia and skin changes in the form of scars on fingers and face as seen in scleroderma. Subsequent follow-up showed predominant features of systemic lupus erythematosus including renal involvement.

Discussion

In 1985, Issit was first to describe AIHA.[8] It results from the development of auto-antibodies directed against antigens on the surface of patient’s own RBCs. Depending on the thermal amplitude of antibodies, it could be cold-antibody or warm-antibody AIHA. Most AIHA cases are due to warm-autoantibodies while cold-autoantibodies are less common.[3] Mixed-AIHA is rare in pediatric patients. Very few reported cases were found on literature review.[4,9]

Differentiation between warm and cold-antibodies in AIHA can be done by monospecific-DAT which also identifies responsible mechanisms. Warm-antibodies

| Table 1: Blood grouping after prewarming and warm saline wash (before steroid therapy) |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Temperature | Forward grouping | Reverse grouping | BG |
| 4°C | Anti A | Anti B | Anti D1 | Anti D2 | A cells | B cells | O cells | AC |
| 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 2+ | 2+ | 2+ | Invalid |
| 22°C | 2+ | 4+ | 4+ | 4+ | 4+ | 2+ | 2+ | 2+ | Invalid |
| 37°C | 1+ | 4+ | 4+ | 4+ | 4+ | 1+ | 1+ | 1+ | Invalid |
| AC=Auto control, BG=Blood group |

| Table 2: Blood grouping after 2 days of steroid therapy |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Temperature | Forward grouping | Reverse grouping | BG |
| 4°C | Anti A | Anti B | Anti D1 | Anti D2 | A cells | B cells | O cells | AC |
| 0 | 4+ | 4+ | 4+ | 4+ | 4+ | 0 | 0 | 0 | B+ |
| 22°C | 0 | 4+ | 4+ | 4+ | 4+ | 0 | 0 | 0 | B+ |
| 37°C | 0 | 4+ | 4+ | 4+ | 4+ | 0 | 0 | 0 | B+ |
| AC=Auto control, BG=Blood group |
show a positive reaction with anti-IgG and negative with anti-C3d, and are usually seen in idiopathic or drug-associated AIHA. If positive reaction is seen with both anti-IgG and anti-C3d, it also indicates warm-autoantibodies but more common in patients with mixed-AIHA. In cold antibody disease, anti-C3d shows a positive while anti-IgG shows a negative reaction.[10] In our case, red cells agglutination at room-temperature reversing on warming and presence of cold-agglutinins at 4°C was seen. However, monospecific-DAT done subsequently showed positivity for anti-IgG and anti-C3d. Based on these findings, a diagnosis of mixed-type of AIHA was proposed. This also emphasizes importance of performing monospecific-DAT.

AIHA is known to be associated with infection, malignancy or autoimmune diseases but mostly idiopathic.[2,3] Corticosteroid is the mainstay therapy. Due to increased requirements for folate, oral folate supplementation is also done. Patients unresponsive to corticosteroids may benefit from chemotherapy and/or splenectomy. Our patient was managed with corticosteroid, hematinsics, and transfusion of packed red cells. Transfusion in AIHA can be complicated because of blood-grouping and cross-matching problems owing to clumping of red cells. We were able to resolve the problem and issued least incompatible B-positive PRBC. No adverse events were encountered during/after transfusion. She improved and is currently on follow-up.

**Conclusion**

We are reporting this case due to rarity of mixed-type AIHA in children. Diagnosis requires a detailed clinical and immunohematological workup. Transfusion should be done judiciously with least incompatible unit under strict clinical supervision. Decision to transfuse should depend on evaluation of patient’s clinical status and assessment of benefits and potential risks of transfusion.

**Acknowledgment**

The authors would like to extend sincere regards to the Department of Pediatrics, All India Institute of Medical Sciences, Raipur (C.G.).

**Declaration of patient consent**

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

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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