CORRESPONDENCE

Blood Group Discrepancy-First Sign of Autoimmune Hemolytic Anemia after Hematopoietic Stem Cell Transplantation in a Child

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Abstract A 12-year-old male child was presented in the emergency with features of anemia and mild icterus on day+67 of HSCT. The child was suffering from Fanconi anemia and undergone HSCT from ABO-matched, fully HLA matched sibling donor. The diagnosis of mixed type AIHA due to cytomegalovirus reactivation was made in the immunohematology laboratory and blood group discrepancy was the first sign of AIHA in this patient. Though the cold agglutinin titer was not significant but the clinical symptoms and laboratory evidences were suggestive of significant hemolysis due to underlying IgG autoantibody. In addition the high complement avidity of IgM autoantibody might also be a contributing factor for clinically significant hemolysis in this case. The patient was successfully treated with phenotype matched blood transfusion, rituximab and oral steroid therapy.

Keywords Hematopoietic stem cell transplantation · Autoantibody · Phenotype matching

To The Editor

Autoimmune hemolytic anemia (AIHA) is a recognized complication of hematopoietic stem cell transplantation (HSCT); it is often refractory to treatment and carries a

high mortality [1]. The overall incidence of AIHA after HSCT is around 3.6 % in adults [2] and 4.4–6 % in pediatric patients [3]. There are three major risk factors associated with autoimmune manifestations in post-HSCT patients-(1) younger age of the patient, (2) diagnosis of non-malignant disease and (3) HSCT from unrelated donor or cord blood transplantation [4]. Patients with nonmalignant disease have a relatively competent immune system prior to HSCT as they have not been exposed to cytotoxic or immunosuppressive therapy. It is possible that a more ‘intact’ immune system during the period of immune reconstitution may contribute to the higher incidence of AIHA in this patient population [5]. The presence of concurrent cold and warm autoantibodies are associated with more severe clinical course in post-HSCT patients [6]. Here we discuss a case in a post stem cell transplant patient where blood group discrepancy was the first sign of AIHA and the diagnosis was made in the immunohematology laboratory.

A 12-year-old male child was suffering from Fanconi anemia and undergone HSCT from ABO-matched, fully HLA matched sibling donor. The conditioning was done by fludarabine (30 mg/m²/day for 6 days) and cyclophosphamide (10 mg/kg/day for 2 days). Total 3×10^6 /kg CD34+ cells were collected by apheresis from peripheral blood of sibling donor and transfused to the patient. Both patient and donor were typed previously as O, D + C-c + e + E + (DcE/Dce) and Kell-negative. There was complete engraftment on day+28. The child was suffering from cytomegalovirus reactivation since day+40 of post-transplant period and was treated successfully with intravenous valganciclovir. He had a history of packed red cell transfusion on day+60 due to anemia probably caused by the valganciclovir induced myelosuppression. He was discharged in a stable condition after transfusion. But on

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day+67 of HSCT he was again readmitted in the emergency with features of anemia and mild icterus. The request was sent to the blood bank for two units of packed red cells. In the blood bank type-IV discrepancy was observed during blood grouping which was subsequently resolved after six times warm saline washing of red cells (Fig. 1) and 60 min incubation of patient's serum at 37 °C. Red cells were showing agglutination at room temperature. Direct antiglobulin test (DAT) with polyspecific reagent revealed 4+ reaction (Ortho Biovue System, Ortho clinical diagnostics, High Wycombe, UK). On further evaluation DAT was reactive to IgG as well as to complement (C3). Antibody screening was performed with three cell panel (Ortho clinical diagnostics, High Wycombe, UK) which showed a panagglutination pattern. Concurrent presence of an IgM autoantibody associated with IgG autoantibody was suspected based on loss of agglutination upon warm saline washing and dithiothreitol (DTT) treatment of red cells [7]. However, cold agglutinin titers revealed maximum agglutination at 4 °C (1:64) with minimum agglutination at 22 °C (1:2) in saline phase. Thermal amplitude test revealed 1+ agglutination at 37 °C (1:2) in AHG phase. To confirm the diagnosis patient's serum was treated with 0.01 (M) DTT at 37 °C for 1 h and titration was performed at three different temperatures (4, 22 and 37 °C) [7]. There was no agglutination seen at 4 or 22 °C after the DTT treatment of the serum. But the reaction persisted as 1+ at 37 °C even after DTT treatment of serum. Additional testing revealed no Donath–Landsteiner antibodies, ruling out paroxysmal cold hemoglobinuria [7]. As the patient had recent history of blood transfusion alloabsorption was performed with R₁R₁, R₂R₂ and rr cells [7, 8] but no red cell alloantibody detected in this case ruling out presence of alloimmunization with the autoantibodies. Other laboratory values on admission were also suggestive of AIHA in this patient: hemoglobin (Hb)—2.9 g/dl, reticulocyte count—17.8 %, unconjugated bilirubin—3.3 mg/dl (0.0–1.1 mg/dl), LDH- 2915 U/l (313–618U/l).

Fig. 1 Resolution of group discrepancy

Based on these results, a case of mixed type of AIHA was suspected in this patient with presence of both IgM and IgG in patient's serum. The probable etiology in this case was cytomegalovirus reactivation in post-HSCT period. A common method of detecting an IgM autoantibody is through DTT treatment. DTT inactivates IgM reactivity by reducing the disulfide bonds found in the tertiary structure unique to the pentameric IgM that are absent in other immunoglobulins. If an IgM autoantibody is present in the patient serum, DTT treatment will abolish any spontaneous RBC agglutination. For this patient, DTT treatment abolished RBC agglutination demonstrating that an IgM autoantibody was present associated with an IgG autoantibody. The cold autoantibody detected in this case had apparent specificity against 'I' antigen as the serum was reacted with the adult pooled O cells only which contains large amount of 'I' antigens over red cell membrane. Though the cold agglutinin titer was not significant ($64 < 1000$) but the clinical symptoms and laboratory evidences were suggestive of significant hemolysis due to underlying IgG autoantibody. In addition the high complement avidity of IgM autoantibody might also be a contributing factor for clinically significant hemolysis in this case. The patient had received two units of phenotype matched red cells O, (DcE/Dce) and Kell-negative to maintain the hemoglobin level and to avoid future alloantibody formation. Phenotype matched RBC units were issued within 2 h after resolution of blood group discrepancy but the entire work-up took around 6 h to complete. He was managed successfully with rituximab (375 mg/m² weekly for 4 weeks) along with oral steroid. The transfusion proceeded without any complications and his Hb level was 7.3 g/dl after successful two units of transfusion along with medications. He was discharged in hemodynamically stable condition and at present he is on regular follow-up.

The underlying pathogenesis of AIHA in the post-HSCT setting is poorly understood but is probably related to immune dysregulation with the majority of patients demonstrating disease refractory to traditional steroid therapy [4, 5]. The profound immune dysregulation may be explained by Daikeler and Tyndall hypothesis of either the imbalance between autoreactive and regulatory lymphocyte population or the loss of self-tolerance mechanisms; the presence of genetic difference in the major or minor HLA genes between the donor and the recipient; the transfer of autoimmunity from the donor to the recipient; and as a direct effect to the conditioning regimen for the HSCT [9]. Infections may be an important trigger for autoimmune events occurring after HSCT. In the study conducted by Sherer et al. [10] 24 % of these viral events were represented by cytomegalovirus reactivations, thus confirming that cytomegalovirus may contribute to the onset of post-transplantation autoimmune complications. In

patients where post-HSCT AIHA is documented, there are no standard recommendations for management that can be made as a result of the vast clinical variability of each patient, including time of post-HSCT, development of hemolysis, presence of GvHD and the status of immunosuppressive therapy at the time of presentation, all of which will impact therapeutic choices [5]. But there are an increasing number of reports on the successful treatment of refractory AIHA with rituximab in post-HSCT settings [4, 11]. Hemoglobin levels may drop to life-threatening levels and the response to standard therapeutic agents are typically quite slow, therefore, transfusion with the phenotype matched red blood cells is frequently necessary and should be considered to avoid delay in transfusion. Additionally, prospective collection of immune reconstitution data for HSCT patients who develop AIHA compared with those who do not may help to evaluate the incidence, complications, and management of post-transplant AIHA.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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