Fatal Immune Hemolytic Anemia Following Allogeneic Stem Cell Transplantation: Report of 2 Cases and Review of Literature

Jordina Rovira a, Joan Cid b,⁎, Gonzalo Gutiérrez-García a, Arturo Pereira b, Francesc Fernández-Avilés a, Laura Rosiñola a, Carmen Martínez a, Enric Carreras a, Álvaro Urbano a, Montserrat Rovira a, Miguel Lozano b

a Department of Clinical Hematology, Hospital Clinic, IDIBAPS, Barcelona, Spain
b Department of Hemotherapy and Hemostasis, Hospital Clinic, IDIBAPS, Barcelona, Spain

ABSTRACT

Immune hemolytic anemia is a well-recognized complication after allogeneic hematopoietic stem cell transplantation (HSCT). There are 4 possible causes for this complication. First, antibodies present in the recipient destroy donor cells. Second, donor red cell antibodies at the time of stem cell infusion are transferred to the recipient. Third, sometimes, engrafted donor lymphocytes cause active production of red cell antibodies. Fourth, another cause of hemolysis after allogeneic HSCT is autoimmune hemolytic anemia (AIHA). It is thought to be due to antibodies produced by the donor's immune system against antigens on red cells of donor origin. Autoimmune hemolytic anemia after allogeneic HSCT is rare, it is still not well characterized, and it represents a life-threatening situation. We describe 2 patients with acute myeloid leukemia treated with intensive chemotherapy and umbilical cord blood stem cell transplantation (UCBT). One patient developed AIHA at day +182 and the other at day +212 after receiving UCBT. Patients received 5 and 7 line treatment options, respectively, including continuous corticosteroids, intravenous immunoglobulin, splenectomy, cyclophosphamide, plasma exchange, rituximab, bortezomib, and eculizumab. However, both patients died because of massive hemolysis after 85 and 106 days of intensive treatment, respectively. These cases reflect the extreme difficulty in the therapeutic management of patients with AIHA following UCBT. After an extensive review of the literature, the exact physiopathologic mechanisms of AIHA after allogeneic HSCT in general, and after UCBT in particular, and therefore an effective treatment remain unknown.

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Case Reports

Case 1

A 26-year-old woman was diagnosed with an acute myeloid leukemia with t(6;9) and ITD-FLT3 in February 2007. This patient was
treated with intensive chemotherapy, achieving a first complete remission (CR) with negative minimal residual disease. Because of her poor prognosis and the fact that she did not have a sibling donor or an HLA-matched unrelated donor, an unrelated 5/6-locus matched donor UCBT was performed. The total nucleated cell and the CD34+ cell counts were 2.8 × 10^7/kg and 1.7 × 10^6/kg, respectively. Major ABO mismatch between the recipient and donor was present (donor: A+; recipient: O+). The patient received a conditioning treatment with total body irradiation, cyclophosphamide (CFM), and antithymocyte globulin. Cyclosporine (CsA) and mycophenolate mofetil (MMF) were given as prophylaxis for graft-vs-host disease (GVHD). The evaluation of the status disease after 3 to 6 months from UCBT showed a CR with negative minimal residual disease and a chimera 100% donor (in granulocytes, not assessable in lymphocytes). During this time, the patient developed multiple cytomegalovirus (CMV) reactivations that required antiviral treatment and acute grade II skin GVHD treated with steroids (prednisone 1 mg/[kg d]) with good response.

The patient developed AIHA at day +182 after UCBT. The analytical data were as follows: hemoglobin 71 g/L, reticulocytes 20.1 × 10^9/L, lactate dehydrogenase 785 IU/L, indirect bilirubin 0.9 mg/dL. The direct antiglobulin test was strongly reactive using anti-IgG and anti-C3d sera. A warm-reacting panspecific IgG was detected in the serum and in the eluate. At that time, ABO red cell test gave a mixed-field pattern of agglutination (A and O group). Before developing AIHA, the patient was still transfusion dependent; and she was receiving transfusion of O+ RBC units every 15 days. A bone marrow aspirate showed erythroblastopenia. Moreover, the IgM and IgG parvovirus B19 serological test results were positive. The patient received treatment with prednison 1 mg/[kg d] without response. Because of the treatment failure with prednison, the patient was also treated with 0.5 g/kg IVIG (12 doses), rituximab 375 mg/m² (4 doses), bortezomib 1.3 mg/m² (4 doses), and 1 dose of eculizumab 600 mg (humanized monoclonal antibody against the complement protein C5). The patient was also splenectomized. None of the multiple therapeutic modalities was effective. Plasma exchange was performed every 48 hours; but at one point, the patient needed it daily. Sixty red blood cell units were transfused, and 39 plasma exchanges were made. The patient was refractory to all treatments, and he died because of massive hemolysis at day +318 after receiving UCBT and after 106 days of intensive treatment. The complete treatment course is depicted in Figure 1.

Discussion

These case reports illustrate that AIHA after receiving unrelated UCBT is a severe complication, even fatal. In fact, severe hemolysis was the cause of death in our 2 patients after long and intensive treatment. These patients died after receiving intensive treatment during 85 and 106 days, respectively. They received 5 and 7 different treatment approaches, and none of them was capable of improving severe

Case 2

A 28-year-old man was diagnosed with acute myeloid leukemia with ITD-FLT3 in August 2010. The patient received intensive chemotherapy as induction treatment and achieved CR, and then an unrelated 4/6-locus matched donor UCBT was performed. The total nucleated cell and the CD34+ cell counts were 1.57 × 10^7/kg and 1 × 10^6/kg, respectively. There was no ABO mismatch between the recipient and donor. The patient was also conditioned with total body irradiation, CFM, antithymocyte globulin, and CsA/MMF for GVHD prophylaxis. The patient presented multiple CMV reactivations that required antiviral treatment. The patient did not present GVHD signs.

The patient developed severe AIHA at day +212 after UCBT. At that moment, the patient was in CR; and the chimera was 100% of donor origin (total leukocytes). The analytical data were as follows: hemoglobin 57 g/L, reticulocytes 8.0 × 10^9/L, lactate dehydrogenase 4782 IU/L, indirect bilirubin 1.3 mg/dL. The direct antiglobulin test was strongly reactive using anti-IgG and anti-C3d sera. A warm-reacting panspecific IgG was detected in the serum and in the eluate. Furthermore, an erythroblastopenia was present; but parvovirus B19 serological test results were negative. The patient received treatment with prednison 1 mg/[kg d]. After previous treatment failure, he was also treated with 0.5 g/kg IVIG (12 doses), rituximab 375 mg/m² (4 doses), bortezomib 1.3 mg/m² (4 doses), and 1 dose of eculizumab 600 mg (humanized monoclonal antibody against the complement protein C5). The patient was also splenectomized. None of the multiple therapeutic modalities was effective. Plasma exchange was performed every 48 hours; but at one point, the patient needed it daily. Sixty red blood cell units were transfused, and 39 plasma exchanges were made. The patient was refractory to all treatments, and he died because of massive hemolysis at day +318 after receiving UCBT and after 106 days of intensive treatment. The complete treatment course is summarized in Figure 2.

Discussion

These case reports illustrate that AIHA after receiving unrelated UCBT is a severe complication, even fatal. In fact, severe hemolysis was the cause of death in our 2 patients after long and intensive treatment. These patients died after receiving intensive treatment during 85 and 106 days, respectively. They received 5 and 7 different treatment approaches, and none of them was capable of improving severe
hemolysis. Both treatment approaches consisted of continuous steroids, IVIG, splenectomy, and rituximab, followed by CFM in one case and plasma exchange, bortezomib, and eculizumab in the other case.

Although autoimmune diseases (AD) are well-recognized complications after allogeneic HSCT, there is not a complete knowledge about the origin and treatment [13,14]. The most important function of the immune system is to differentiate between self and nonself structures. Nowadays, it seems that homeostatic expansion after allogeneic HSCT lymphopenia could trigger a loss of self-tolerance and proliferation of autoreactive T cells that are the causative agents for AD. However, the detailed mechanisms in humans remain unclear; and despite recent advances in our understanding of the process, autoimmunity remains largely a black box [15,16].

The frequency of AD after allogeneic HSCT is low; and current data arise mainly from single cases, as in the present study, and single-center, retrospective studies [16]. Autoimmune cytopenias are the most commonly encountered AD in this setting. Autoimmune hemolytic anemia was described with an overall incidence of 3% in adults [3] and 6% in children [5]. One must keep in mind the difficulty in determining the origin of the antibodies. As defined previously, antibodies of donor origin against antigens on donor cells or antibodies of recipient origin against antigens on the recipient’s red cells may be considered autoantibodies [1].

The most important risk factors associated with the development of AIHA after allogeneic HSCT were the unrelated donor and the presence of chronic GVHD, probably reflecting increased defective T-cell function [6,13,17]. Several publications have suggested that AIHA might be a form of chronic GVHD [4,18]. The recipient pretreatment serologic status of CMV has also been associated with GVHD and may potentially be associated with the development of AD [4,19]. On the contrary, the T-cell depletion has not been a significant variable in the development of AIHA [5,6,13,20]. Some authors have suggested a pathogenic role of CsA in AIHA in terms of dysregulated T cells. However, in the literature, there are some case reports that only responded to CsA and other AIHA cases that did not receive it; as a result, this is a controversial point to be further analyzed [3,15]. The main characteristics of published AIHA after HSCT cases to date are summarized in Table.

Some authors reported the indication for allogeneic HSCT in nonmalignant disease as the risk factor for post-HSCT AIHA in a pediatric cohort [5]. Interestingly, a retrospective single-center study reported a high incidence of autoimmune cytopenias (10/19) in children after UCBT for congenital diseases [33] and other case reports described AIHA after UCBT [18,24,32,34]. Although the majority of published cases were referred to AIHA after UCBT, this correlation has not been well analyzed [18,24,32]. In fact, AIHA after UCBT seems to be more common in pediatric patients because it is more frequently used in children than in adults. Radhi et al [8] described a patient with familial hemophagocytic lymphohistiocytosis cured by UCBT who later developed a complication of severe AIHA treated and solved with rituximab. Rokicka et al [34] reported the only published case of AIHA developing after double UCBT. A case of Evans’ syndrome after UCBT has also been published [31]. Some authors have postulated that AIHA following UCBT in very young infants (≤3 months of age) is due to aberrant immune ontogeny associated with GVHD prophylaxis during the first year of life [33]. With increasing use of cord blood as a source of stem cells, this has to be further investigated. In fact, in our center, after the experience with the 2 patients reported in the present study, we have decided to prospectively follow the immunohematologic complications of patients after receiving HSCT to gain more knowledge.

The median time to diagnosis of AIHA after HSCT varies between 1 and 6 months. In this context, different authors have postulated the notion that cold agglutinin-mediated (IgM) hemolysis occurs earlier than IgG ones (2-8 months after HSCT vs 6-18 months) parallel to the reconstitution of the donor immune system [3,22]; however, this concept has not been confirmed in other publications [6]. Patients with both warm and cold autoantibodies are rarely seen, but they

### Table

Published data about AIHA after allogeneic HSCT

<table>
<thead>
<tr>
<th>Author [reference]</th>
<th>Publication</th>
<th>Patients</th>
<th>Stem cell source</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sokal et al [21]</td>
<td>1987</td>
<td>1</td>
<td>BM</td>
<td>AIHA and virus-associated hemophagocytic syndrome</td>
</tr>
<tr>
<td>Bashey et al [7]</td>
<td>1991</td>
<td>1</td>
<td>BM</td>
<td>Late onset immune pancytopenia in the absence of chronic GVHD</td>
</tr>
<tr>
<td>Tamura et al [22]</td>
<td>1994</td>
<td>1</td>
<td>BM</td>
<td>Cold agglutinin disease following HSCT resolved with steroids</td>
</tr>
<tr>
<td>De Lord et al [9]</td>
<td>1996</td>
<td>1</td>
<td>BM</td>
<td>Comitant AIHA, ITP, and granulocyte-specific antibodies</td>
</tr>
<tr>
<td>Drobyski et al [15]</td>
<td>1996</td>
<td>7</td>
<td>BM</td>
<td>AIHA post-HSCT is a refractory disease; higher incidence in ex vivo T-cell–depleted allogeneic HSCT (5%)</td>
</tr>
<tr>
<td>Chen et al [3]</td>
<td>1997</td>
<td>9</td>
<td>BM</td>
<td>3.1% incidence in adults; cold autoantibodies appeared 2-8 mo after HSCT, whereas warm antibodies appeared at 6-18 mo</td>
</tr>
<tr>
<td>Godder et al [4]</td>
<td>1997</td>
<td>5</td>
<td>BM</td>
<td>7.5% Coombs positive AIHA in T-cell–depleted HSCT</td>
</tr>
<tr>
<td>Horn et al [23]</td>
<td>1999</td>
<td>8</td>
<td>4 BM &amp; 4 PB</td>
<td>High incidence in complete T-cell–depleted HSCT</td>
</tr>
<tr>
<td>Mullen et al [24]</td>
<td>2000</td>
<td>1</td>
<td>UCB</td>
<td>Pediatric unrelated umbilical cord blood transplantation for Hunter syndrome; treated with prednisone and tacrolimus</td>
</tr>
<tr>
<td>Avu et al [25]</td>
<td>2000</td>
<td>1</td>
<td>BM</td>
<td>Fulminating late-onset cold hemagglutinin disease after HSCT</td>
</tr>
<tr>
<td>Cwynarski et al. [20]</td>
<td>2001</td>
<td>9</td>
<td>BM</td>
<td>Lymphocyte infusion is an effective therapy for AIHA after HSCT in context of leukemia relapse</td>
</tr>
<tr>
<td>Hartert et al [10]</td>
<td>2001</td>
<td>1</td>
<td>PB</td>
<td>AIHA after HSCT resolved with IVIG</td>
</tr>
<tr>
<td>Pratt et al [26]</td>
<td>2001</td>
<td>1</td>
<td>BM</td>
<td>Hemolysis resolved after 4 y of continuing immunosuppressive therapy</td>
</tr>
<tr>
<td>Sevilla et al [18]</td>
<td>2001</td>
<td>1</td>
<td>UCB</td>
<td>AIHA following cord blood transplantation; AIHA as the first sign of chronic GVHD</td>
</tr>
<tr>
<td>Hongeng et al [27]</td>
<td>2002</td>
<td>1</td>
<td>BM</td>
<td>Resolved with rituximab after steroid and IVIG refractoriness</td>
</tr>
<tr>
<td>Ship et al [28]</td>
<td>2002</td>
<td>1</td>
<td>PB</td>
<td>AIHA after HSCT resolved with rituximab</td>
</tr>
<tr>
<td>Corti et al [29]</td>
<td>2003</td>
<td>1</td>
<td>PB</td>
<td>Rituximab for AIHA following T- and B-cell–depleted HSCT</td>
</tr>
<tr>
<td>O'Brien et al [5]</td>
<td>2004</td>
<td>19</td>
<td>10 BM &amp; 9 UCB</td>
<td>6% incidence in children; mortality as high as 50%</td>
</tr>
<tr>
<td>Raj et al [30]</td>
<td>2005</td>
<td>4</td>
<td>PB</td>
<td>First time reported autoimmunity disorders after reduced-intensity conditioning HSCT; efficacy of rituximab</td>
</tr>
<tr>
<td>Chen et al [31]</td>
<td>2007</td>
<td>1</td>
<td>UCB</td>
<td>Evans syndrome after cord blood transplantation treated with IVIG, steroids, vincristine, cyclosporine, and mycophenolate mofetil.</td>
</tr>
<tr>
<td>Kako et al [12]</td>
<td>2007</td>
<td>1</td>
<td>PB</td>
<td>AIHA and PReA simultaneously after HSCT</td>
</tr>
<tr>
<td>Radhi et al [8]</td>
<td>2007</td>
<td>1</td>
<td>UCB</td>
<td>AIHA + ITP after cord blood transplant steroid refractory but responded to rituximab</td>
</tr>
<tr>
<td>Sanz et al [6]</td>
<td>2007</td>
<td>12</td>
<td>3 BM &amp; 5 PB &amp; 4 UCB</td>
<td>4.4% incidence in adults</td>
</tr>
<tr>
<td>Chao et al [32]</td>
<td>2008</td>
<td>1</td>
<td>UCB</td>
<td>Successful treatment with Campath-1H</td>
</tr>
<tr>
<td>Page et al [33]</td>
<td>2008</td>
<td>4</td>
<td>UCB</td>
<td>High incidence of autoimmune cytopenias in children (&lt;3 mo of age) after cord blood transplantation for congenital disorders</td>
</tr>
<tr>
<td>Rokicka et al [34]</td>
<td>2009</td>
<td>1</td>
<td>UCB</td>
<td>AIHA after double cord blood transplantation</td>
</tr>
</tbody>
</table>

BM: bone marrow; PB: peripheral blood; UCB: umbilical cord blood; ITP: immune thrombocytopenic purpura.
have the most severe clinical course [23,25]. In some reports, some antibodies seem to have an apparent specificity [15,23]; but there are several cases with a nonspecific or panreactive component that is typical of AIHA [3,31]. The apparent specificities of the antibodies were against the antigens shared by both donor and recipient [3]. Regarding therapeutic options, the steroids are usually the first-line treatment despite knowing that AIHA after HSCT often is refractory to this traditional therapy. The treatment of steroid refractory cases is not yet well established. In this sense, different single and combined immunosuppressive therapies such as CsA, CFM, vincristine, rituximab, MMF, alemtuzumab, and pentostatin are used. However, all of them lead to severe infection in allogeneic HSCT recipients. Moreover, the IVIG and plasma exchanges are alternative treatments for these patients to allow time for the other treatments to take effect. Red blood cell transfusions are needed throughout the process [16,27-30,35].

The overall response to therapy is generally unsatisfactory. In the majority of cases, a high mortality is present due to infectious complications [5] or, more rarely, by hemolysis itself, which is the case of our 2 patients. It is not clear whether immunosuppressive therapy altered the natural history of the disease or whether the hemolysis slowly improved on its own accord. Autoimmune hemolytic anemia in the setting of chronic GVHD appears to respond within a month to immunosuppressive treatment [4]. Pratt et al [26] reported one patient with AIHA after HSCT who resolved hemolysis after 4 years of continuing immunosuppressive therapy.

Another AD complication is pure red cell aplasia (PRCA) following HSCT, which has been mostly reported in ABO incompatibility situations between donor and recipient [36,37]. The mechanism of PRCA following major ABO-matched allogeneic HSCT remains unclear. To our knowledge, there is only one report with concurrent AIHA and erythroblastopenia [12]. Kako et al [12] reported a case of AIHA and PRCA after HSCT related with the administration of progabide that did not improve after drug discontinuation. This patient had also been treated with alemtuzumab before HSCT. Pure red cell aplasia cases after administration of alemtuzumab have been reported without receiving allogeneic HSCT, most of them in terms of parvovirus B19 infection that developed because of persistent immunosuppression. Autoimmune hemolytic anemia improved with steroids and PRCA with cyclosporine; and that reflects that AIHA might be caused by humoral immune response, whereas PRCA might be mainly caused by cell-mediated immune response. In our first case report, PRCA could have been caused by parvovirus B19 infection; but the PRCA etiology in the other case was unknown, although it might be speculated that it could be provoked by the fact that the antibody recognizes an antigen present early in the red blood lineage and the binding of the antibody would cause the immune destruction. A similar situation is found in the hemolytic disease of the newborn where the disease caused by anti-Kell is mainly erythroblastopenic with little bilirubin increase, whereas, in general, the disease caused by anti-D is mainly jaundice with a significant increase in circulating erythroblasts [38].

Conclusion

The autoimmune cytopenias, such as AIHA, after receiving an allogeneic HSCT are per se life threatening with significant morbidity and mortality. Hemolysis is often severe and chronic, persisting for months or years [19]. Currently, the management of these patients is very difficult because the exact mechanisms of AIHA after allogeneic HSCT and an effective treatment remain to be known.

References


