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**Autologous peripheral blood stem cell transplantation and anti-B-cell directed immunotherapy for refractory auto-immune haemolytic anaemia**

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**Abstract** We report the clinical course of a 6.5-year-old boy with refractory auto-immune haemolytic anaemia. Due to failure of conventional immunosuppressive therapy, an autologous peripheral blood stem cell transplantation was performed. The conditioning regimen consisted of cyclophosphamide and anti-thymocyte globulin. The patient was reinfused with 2.6 × 10⁸ CD34 positive selected, B- and T-cell-depleted peripheral blood stem cells per kg body weight. He showed a partial response with a reduced demand for red blood cell transfusions. However, due to persistence of the haemolytic process he was started on rituximab therapy on day +40 post-transplant. Following two doses of rituximab, the patient improved rapidly and developed a sustained complete response. After 10 months, haemolysis recurred and responded again to rituximab therapy without the necessity for red blood cell transfusions. 15 months after initial antibody treatment, however, the patient developed a second relapse which was now refractory to rituximab therapy although CD20+ B-lymphocytes were cleared from the peripheral blood.

**Conclusion** Our case report suggests that rituximab and autologous peripheral blood stem cell transplantation are important though not curative elements in the treatment of patients with severe auto-immune haemolytic anaemia who are refractory to conventional immunosuppressive therapy.

**Key words** Auto-immune haemolytic anaemia · Autologous peripheral blood stem cell transplantation · Evans syndrome · Immunotherapy · Rituximab

**Abbreviations** AIHA auto-immune haemolytic anaemia · CMV cytomegalovirus · IVIG intravenous immunoglobulins · PBSC peripheral blood stem cells · PBSCIT peripheral blood stem cell transplantation · RBCT red blood cell transfusions

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**Introduction**

The association of auto-immune haemolytic anaemia (AIHA) with immune thrombocytopenic purpura has been known for many years and was first described by Fisher [9] and Evans et al. [7]. In AIHA, patients produce antibodies against erythrocytes which are directed towards membrane glycoproteins or immune complexes adsorbed onto the erythrocyte surface [18]. The aetiology/pathology is still unknown. Drugs, viral infections and/or immunodeficiency have been implicated in triggering auto-immunity [2, 18]. The development of auto-immune cytopena has also been described in connection...
with bone marrow transplantation [12] and occurs in 5% of all patients transplanted with T-cell-depleted stem cells [6]. Steroids are the treatment of first choice, followed by intravenous immunoglobulins (IVIG) and cyclosporine. Danazol, cytotoxic drugs, splenectomy and splenic irradiation have alternatively been applied [5].

In 1997, first clinical data suggested that autologous peripheral blood stem cell transplantation (PBSCT) following intensive immunosuppressive conditioning therapy may be a successful treatment option for selected patients with severe auto-immune disease who have failed conventional therapy [15]. However, despite increasing experience and improved supportive care, the transplant-related mortality rate in patients with auto-immune disease is still as high as 9% within the 1st year [3]. Experience with autologous PBSCT in AIHA is limited. Allogeneic stem cell transplantation, on the other hand, is associated with a high transplant-related mortality. One problem lies in the iron overload and the consecutive risk for toxicity following myeloablative conditioning. In 1997, Raetz et al. [17] published a case report of a 5-year-old boy with severe Evans syndrome. He achieved complete remission after an HLA-identical sibling cord blood transplant. However, he died of acute hepatic failure 9 months after transplantation.

Production of anti-erythrocyte auto-antibodies is fundamental for auto-immune haemolysis suggesting that AIHA is a predominantly B-cell-mediated disease. In 1998, results of a multicentre study revealed that the use of the anti-CD20 antibody rituximab is effective in the treatment of low-grade B-cell lymphomas [16]. This antibody binds to CD20+ cells of the B-lineage leading to their destruction. However, only preliminary data have so far been published on the application of this antibody for non-malignant B-cell disorders.

Here, we describe and discuss an autologous PBSCT followed by splenectomy and treatment with the anti-CD20 antibody rituximab in a child with refractory AIHA. The role of rituximab in conditioning regimens of autologous stem cell transplantsations for auto-immune diseases and in post-transplant consolidation therapy will be discussed.

Case report

A 6.5-year-old boy developed AIHA after an upper respiratory tract infection in June 1997. Despite immunosuppressive therapy, he had a progressive need for red blood cell transfusions (RBCT) exacerbated by frequent upper respiratory infections. Between November 1997 and April 1998 he also developed a transfusion-dependent thrombocytopenia with intermitent platelet counts below 10,000/µl and thus the diagnosis of Evans syndrome was made. Since April 1998, his platelet counts had been stable (>100,000/µl).

Immunosuppressive therapy consisted of steroids (initially prednisolone 10 mg/kg per day for 3 days, followed by 1 mg/kg per day for several weeks) in combination with IVIG (10 g/day for 4 days). He continued to receive RBCT as needed. With hepatosplenomegaly, mild abnormal liver function tests (GPT 56 U/l) and the need of six RBCT per week, the patient was transferred to our hospital in June 1998 for further evaluation and treatment.

At time of admission to our hospital, his laboratory tests showed ongoing haemolysis with a lactate dehydrogenase of 744 U/l and a total bilirubin of 6.0 mg/dl. A positive direct Coombs test and the detection of IgG auto-antibodies directed against the erythrocyte membrane glycoproteins c and C3d confirmed the diagnosis of AIHA. Bone marrow cytology (June 1998) showed marked hyperplasia of the erythroid lineage (70% of all nucleated cells) with only mild dysplasia compatible with severe haemolytic anaemia. There was a normal marrow blast cell count (1%) and no significant dysgranulo- or dysmegalakaryopoiesis. Due to the lack of evidence for a malignant disease no cytogenetic analysis was performed. He was started on an intensive immunosuppressive regimen containing cyclosporine (Sandimmun Optoral, 150 mg/m² per day for 4 weeks; serum level monitoring by monoclonal antibody detection assay: 150–200 ng/ml), five cycles of cyclophosphamide (Endoxan, 750 mg/m²) over a period of 5 months and steroids (methylprednisolone, Uraboron, 20 mg/kg for 3 days every 3–4 weeks). Despite intensive immunosuppression, more than two RBCT per day were necessary to maintain a haemoglobin level above 6.0 g/dl.

Due to the frequent RBCT he became severely iron-overloaded. His ferritin level rose from 2454 µg/l on referral to our hospital to 19280 µg/l leading to a reduced hepatic function with a plasma cholinesterase level of 1200 U/l. A left diastolic ventricular dysfunction and pericardial effusion were interpreted as signs of cardiac iron overload. No endocrinological dysfunction was evident.

In addition to the signs of haemochromatosis, the patient required several empirical antibiotic therapies for fever of unknown origin. Most antibiotic regimens contained an aminoglycoside and a third-generation cephalosporine and were continued for a minimum of 7 days. He also suffered from frequent viral infections, in particular Epstein-Barr virus and cytomegalovirus (CMV) re-activations. CMV re-activations were treated with ganciclovir (Cymeven, 2 x 5 mg/kg per day for 14 days, followed by 5 mg/kg per day Monday to Friday for an additional 3–4 weeks). Response to therapy was monitored by semiquantitative CMV polymerase chain reaction and CMV antigenemia.

Due to the failure of conventional immunosuppressive therapy, increasing haemochromatosis and frequent infections, an autologous PBSCT was considered as a experimental treatment option. Peripheral blood stem cells (PBSC) were mobilised with 10 µg/kg per day G-CSF (Neupogen) subcutaneously. PBSC were harvested on two consecutive days and 2.6 x 10⁸ CD34+ cells/kg body weight (after purging) were collected. The mobilised PBSC were CD34+ selected with simultaneous depletion of B- and T-lymphocytes by a double positive purging. After CD19+ CD22+ + T-cells/kg body weight, 6 x 10¹⁰ CD19+ CD22+ + B-cells/kg body weight. The patient was conditioned with cyclophosphamide (Endoxan, 50 mg/kg/day) from day −5 to −2 and anti-thymocyte globulin (Thymoglobulin Merieux, 3 mg/kg/day) from day −5 to −2 according to published guidelines [22]. On day 0 (March 3, 1999) the purged PBSC were re-infused. The patient was started on G-CSF (10 µg/kg) on day +1.

Empiric antibiotic treatment with meropenem (Meromon) and amikacin (Biklin) was continued until engraftment. Pneumocystis carinii pneumonia prophylaxis consisted of oral trimethoprim (Kepinol, 10 mg/kg twice per week) following engraftment. The post-transplantation course was complicated by a CMV re-activation as detected by CMV antigenemia on day +7 and was treated with ganciclovir according to standard protocol. Engraftment (absolute neutrophil count > 500 x 10⁹) occurred on day +10.

The requirement for RBCT decreased to one transfusion every 2nd day. Because of multiple spleen infarctions, the patient was splenectomised on day +32. Despite clinical improvement, haemolysis as documented by a positive direct Coombs test and the, although reduced, ongoing need for RBCT persisted. It was assumed that auto-antibody-producing B-cell clones had survived the conditioning regimen. Therefore a treatment attempt was made on day +40 using the antibody rituximab (Mabthera, 375 mg/m² body surface area i. v., weekly, for a period of 4 weeks) that recognises the B-cell epitope CD20. Rituximab infusion was well