Successful treatment of pure red cell aplasia with repeated, low doses of rituximab in 2 patients after ABO-incompatible allogeneic haematopoietic stem cell transplantation for acute myeloid leukaemia

We describe two patients with acute myeloid leukaemia (AML) successfully treated with anti-CD20 antibody for pure red cell aplasia (PRCA) following ABO mismatched allogeneic haematopoietic stem cell transplantation (HSCT). PRCA following HSCT is associated with major ABO incompatibility between donor and recipient and is due to an inhibition of donor erythroid precursors by residual host isoagglutinins. The first patient developed PRCA resistant to several treatment options including donor-derived leukocyte infusions (DLI), high-dose erythropoietin (EPO), rapid tapering of cyclosporin A (CsA). This patient also received anti-viral therapy as CMV and parvovirus B19 infections were regarded as additional causes of PRCA. Due to a loss of donor chimaerism, he underwent second HSCT, but PRCA still persisted. He showed no evidence of graft versus host disease (GVHD). Finally he was administered anti-CD20 antibody (rituximab) at dose 150/m2 and PRCA resolved in a short period of time. The case was complicated by life-threatening pulmonary aspergillosis with septic shock, successfully treated with anti-fungal therapy. The second case concerns a patient, who revealed PRCA after major ABO-incompatible HSCT from his brother. Regarding our experience with previous described patient, he was proceeded to administer rituximab at dose 150/m2 as a first line treatment. We observed rapid recovery from PRCA without any side effects. We conclude that rituximab seems to be a promising therapeutic option in patients with PRCA after ABO mismatched HSCT, who failed the conventional treatment.

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Pure red cell aplasia following ABO-incompatible allogeneic HSCT is associated with interaction of recipient anti-A or anti-B isoagglutinins with donor erythroid precursors expressing A and/or B antigens, ABO incompatibility occurs in up to 20-40% of HLA matched allogeneic HSCT, but it does not influence the frequency of graft failure or GVHD. Appropriate treatment of PRCA is not clear. Plasma exchange and immunoadsorption are regarded as a first-line treatment strategy. The other therapeutic approaches, which seem to be effective in some patients include: EPO, DLI, steroids or induction of GVHD by CsA discontinuation. Rituximab (Mabthera; Roche, Switzerland) is a chimeric IgG1 monoclonal antibody directed against the CD20 surface antigen expressed by most human B lymphocytes. It demonstrates a high clinical effectiveness, but its exact mechanism of action is unknown. Some data suggest that rituximab induces apoptosis, complement-mediated lysis and antibody-dependent cellular cytotoxicity. The therapeutic indications include indolent and aggressive B-cell lymphomas, and autoimmune and other diseases. We describe two cases of PRCA after major ABO-incompatible HSCT for acute myeloid leukaemia resolving after repeated, low doses of rituximab.

Study design
Patient 1

A 54-year old male with acute myeloid leukaemia (AML), subtype M2 according to FAB, underwent allogeneic peripheral blood stem cell transplantation (PBSCT) from HLA matched, 59-year old sister in April 2003. As nonmyeloablative conditioning we administered: busulphan (Myleran; Glaxo Wellcome, United Kingdom) 6 mg/kg, fludarabine (Fludara; Schering AG, Germany) 125 mg/m2 and antithymocyte globulin (ATG; Fresenius, Germany) at total dose 1200 mg. GVHD prophylaxis consisted of CsA (Sandimmun Neoral; Novartis, Switzerland). There was major ABO incompatibility between the recipient (0 Rh minus) and donor (A Rh plus). Anti-A isoagglutinin titers of the patient performed before transplantation were 1:32 (IgM) and 1:64 (IgG). The patient received PBSCT following positive CD34+ cells selection on Clnimacs. A total of 0.12x10/kg mononuclear cells, including 3.11x10/kg CD34+ and 0.5x10/kg CD3+ cells were transplanted. Granulocyte engraftment >0.5x10/L was achieved on day +21, platelet >50x10/L occurred on day +40, while anaemia persisted with reticulocytopenia. During the transplantation period, patient received 1600 mL of red blood cells (RBCs), group 0. Bone marrow examination performed on day +30 did not reveal any erythroid precursors, but neutrophils and megakaryocytes were present. The diagnosis of PRCA was set after exclusion of RBC alloantibodies, haemolysis, viral or bacterial infection or relapse. There was mixed myeloid donor chimaerism on day +28. It was assessed using two methods: 1) polymerase chain reaction (PCR)- amelogenin gene and 2) short tandem repeats (STR) polymorphic between the patient and donor. Patient showed no evidence of GVHD. Anti-A isoagglutinin titers on day +28 were 1:16 (IgM) and 1:32 (IgG). On days +29 and +74 we infused donor lymphocytes (DLI) at dose 0.5x10/kg CD3 cells and 1.0x10/kg CD3 cells respectively, but patient was still transfusion dependent. The reason for DLI was to reinforce the impact of graft-derived immunocompetent cells in overcoming the host’s residual lymphocytes, which are responsible for isoagglutinin production and erythroid blockage. There was 95% of donor chimaerism on day +77. CsA was tapering and finally ceased on day +91. In September 2003 we detected parvovirus B19 infection and patient was administered 0.5g/kg intravenous immunoglobulin (Gammmagard; Baxter, Germany). The diagnosis of parvovirus B19 was performed by means of serologic methods on peripheral blood (PB) cells. Soon after the therapy with intravenous immunoglobulin, we observed only transient increase in reticulocytosis up to 5% and 34% of recipient’s erythrocytes possessed donor antigen A. RBC chimaerism was measured by means of flow cytometry. It was performed 3 weeks after administration of immunoglobulin for parvovirus B19 and 6 months after PBSCT. Within next month reticulocytopenia appeared again and patient needed transfusions every three weeks. Anti-A isoagglutinin titers were 1:2 (IgM) and 1:2 (IgG). In a meantime he was given EPO (NeoRecormon; Roche, Switzerland) at dose 10000 IU sc three times a week between days 151 and 179. On control bone marrow biopsy we did not find any erythroid precursors and there was 85% of recipient’s chimaerism on day +180. Patient lost the graft and underwent the second, unmanipulated allogeneic PBSCT from his HLA-matched sister in January 2004. Conditioning consisted of treosulphan (Ovasta; Medac, Germany) -25g/m2 and cyclophosphamide (Endoxan; Baxter, Germany) -30 mg/kg. We transplanted 10.41x10/kg of mononuclear
cells including 6.31×10^7/kg CD34+ cells and 9.47×10^7/kg CD3+ cells. GVHD prophylaxis consisted of cyclosporin A. Granulocytes exceeded >0.5×10^9/L on day +19 and platelets exceeded 50×10^9/L on day +28 while reticulocytes did not exceed 1.5% and anaemia still persisted. During the second HSCT ~3600 mL of RBCs group 0 were transfused. On bone marrow examination we found no erythroid cells. Patient did not develop GVHD. There was complete myeloid donor chimaerism on day +29. Anti-A isoagglutinin titers were 1:2 (IgM) and 1:2 (IgG). Patient was transfusion dependent. He was also given EPO after 2nd PBSCT. On day +62 we detected recurrent parvovirus B19 infection and patient was treated with immunoglobulin at dose 200 g. Additionally we detected cytomegalovirus (CMV) infection on PCR and 21-day therapy with gancyclovir (Cymevene; Roche; Switzerland) at dose 10 mg/kg was administered. CsA was ceased on day +90 after second PBSCT. Due to persisting anaemia we started the therapy with rituximab at dose 150 mg/m^2 once weekly for a total of 3 doses between days +94 and +108 (total dose 900 mg). On flow cytometry we observed a total depletion of lymphocytes B with resolution of PRCA on day +133 after second HSCT. On bone marrow examination we revealed increasing number of erythroid cells, reticulocytosis increased up to 10% and patient became transfusion independent. Treatment with rituximab was complicated by pulmonary aspergillosis, successfully treated with amphothericin B (Fungizone; Bristol Myers Squibb, United Kingdom) and voriconazole (Viendi; Pfizer, Germany). Currently patient is in overall good condition, with normal blood parameters and complete donor chimaerism.

**Patient 2**
Second patient was a 47-year old male with a 4 month history of acute myeloid leukaemia (AML-M1). In April 2004 being in first complete remission, he received allogeneic bone marrow transplantation from his HLA matched 39-year old brother. As conditioning he was given busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg), GVHD prophylaxis consisted of CsA and short-course methotrexate (Methotrexate; Pliva, Czech Republic) There was major ABO mismatch between donor (group B Rh minus) and patient (group 0 Rh plus). Pretransplant anti-B isoagglutinin titers were 1:2 (IgM) and 1:2 (IgG). We transplanted 200 mL of graft including 0.26×10^7/kg of mononuclear cells, 0.27×10^7/kg CD34+ and 0.78×10^7/kg CD3+ cells. Within next week, due to small number of transplanted cells, patient underwent unmanipulated PBSCT and received 5.85×10^7/kg of mononuclear cells including 4.43×10^7/kg CD34+ and 14.8×10^7/kg CD3- cells. Peripheral blood stem cells were mobilized from the donor with G-CSF at dose 0.48mg sc for 5 days. Patient did not develop GVHD. Granulocytes engraftment >0.5×10^9/L occurred on day +31 and platelets achieved >50×10^9/L on day +22. Reticulocytes did not exceed 1.5%. There was deep anaemia and patient was transfusion dependent. On bone marrow exam performed on day +30 we did not find any erythroid precursors. Anti-B isoagglutinin titers were 1:4 (IgM) and 1:4 (IgG). PRCA was diagnosed after exclusion of viral infections (CMV, EBV, parvovirus B19), haemolysis, alloantibodies and relapse. There was complete myeloid donor chimaerism. As a first-line therapy we administered rituximab- 150 mg/m^2, once weekly for a total of 3 doses. As a supportive therapy patient was given folic acid, vitamin B12 and EPO at dose 10000IU sc every 2-day for 4 weeks. Patient 2 resolved PRCA in a short period of time; 10 days after the last rituximab dose (+10), his reticulocytosis exceeded 3.5% and hemoglobin level achieved 8.0 g/dL (before rituximab therapy hemoglobin level was 6.4 g/dL). On bone marrow exam performed on day +35, we revealed 47% of erythroid cells. Currently, 8 months after the therapy with rituximab, hemoglobin level is as high as 13.0 g/dL with reticulocytosis exceeding 9%. Patient is transfusion independent and PRCA free.

**Discussion**
PRCA following ABO-incompatible alloSCT results from persistence of host-derived lymphocytes or plasma cells which have survived after conditioning regimen and produce isoagglutinins directed against donor erythroid precursors. Some reports indicate that PRCA can resolve without any therapeutic approaches by decreasing of isoagglutinin titers <1:16, but majority of patients require therapy. It has been shown, that high level of pre-transplant isoagglutinin titers (both anti-A/anti-B) strictly correlate with delay in erythropoiesis onset after SCT, but some authors did not share that view.

Our patients received RBC-depleted marrow, but did not receive pre-transplant plasma exchange. In first described patient pre-transplant isoagglutinin titers were slightly increased to 1:32 and post-transplant titer remained <1:16 during follow-up, however no erythroid precursors were found on bone marrow exam. The isoagglutinin titers in the second case did not exceed 1:2 in pre- transplant period and 1:4 after HSCT. We did not find any other explanation for erythroid engraftment failure, so PRCA appears to be associated with ABO mismatch. In addition, our patients revealed several risk factors for the development of PRCA. Both of them received transplant from sibling donors and cyclosporine A was administered for GVHD prophylaxis. The risk of PRCA in first patient was increased by the presence of anti-A agglutinins and by nonmyeloablative conditioning.

In recent published studies it was shown that rituximab can be effective in a number of patients with immune-mediated syndromes such as: haemolytic anaemia. There is also the description of clearance of red blood cell alloantibodies using rituximab. The recovery of PRCA with a single dose of anti-CD20 antibody (200 mg/m^2) in a child after ABO-mismatched alloSCT for aplastic anaemia was reported by Maschan and colleagues. One single case of adult patient with PRCA after major ABO-incompatible PBSCT for leukaemia resolving after 4 doses of rituximab at dose 235 mg/m^2 has been recently described. We started the therapy with rituximab at lower dose- 150 mg/m^2 once a week over 3 weeks. In a short period of time we observed a spectacular recovery of PRCA. Regarding that effective therapy, the second patient with PRCA was proceeded to administer rituximab at the same dose- 150 mg/m^2 as a first line therapy and soon thereafter, PRCA resolved. In that case isoagglutinin titers were on very low level, both in pre- and post-transplant period. The rapid increase in reticulocyte count and subsequent PRCA recovery in a few days after rituximab infusions was associated with almost complete CD19+ cells depletion from peripheral blood. The lymphocyte population was measured after rituximab infusions and B-cell recovery for patient 1 started from 7 months and was complete after about 10 months. B-cell recovery was shorter for patient 2 and started from 5 months and was complete after about 8 months. B-cell depletion can be dose-dependent, and it seems to be shorter after lower dose of rituximab if compare to conventional one. Immunoglobulin serum level
(IgG, IgM, IgA) was normal before rituximab therapy and remained normal after treatment, but in the lower normal range. Both presented patients did not receive intravenous immunoglobulin as a replacement therapy.

In our patients we administered lower doses of rituximab than those needed for treatment of lymphomas and it appeared to be effective in eliminating anti-donor isoagglutinin from recipient’s blood. Since rituximab administration could result in infectious complications and in fact, we observed a severe, life-threatening pulmonary aspergillosis, the included patients should be selected carefully. Only one month after the 3rd dose of rituximab, patient 1 was admitted to the hospital in overall bad condition, with fever and severe dyspnoe. He required oxygen as an initial therapy. One week later patient revealed septic shock and amines must have been administered to increase his blood pressure. Chest X-ray and computed tomography (CT) of the lungs revealed pulmonary infiltrations which have been assessed by radiologists as characteristic for fungal infection. The diagnosis was confirmed by positive galactomannan test using ELISA. The mechanism by which treatment with rituximab is effective in PRCA after ABO-mismatched HSCT is poor known and requires further studies. We observed an early, but long-lasting response to rituximab treatment. Patient 1 resolved PRCA after 3 weeks and patient 2 started to recover the erythropoiesis merely about 10 days after rituximab therapy. There was no need to infuse rituximab as a maintenance therapy even then, when B-cell reconstitution was complete. It seems unlikely that a rapid drop of isohemagglutinin titters directed against donor-derived erythroid progenitors is responsible for PRCA resolution in presented patients. They were remaining low, both before transplantation and also in post-transplant period. Rituximab can be effective in the treatment of immune-mediated disorders such PRCA or ITP with 2 different mechanisms. We can assume that not only one was involved in PRCA recovery in our patients. An early (rapid) phase of response was probably due to opsonized B-cells that can inhibit macrophage Fe-receptor function. The suppression of autoreactive B-cells responsible for the production of autoantibodies can serve as an additional mechanism which enables to sustain remission.5,23 Since we know, that early red blood cells precursors express blood group antigens to a lesser extent and A (or B) antigen was shown to be on almost 50% of BFU-E and 83% of CFU-E cells, it is likely that host-derived isoagglutinins interact with donor’s erythroid progenitors within the bone marrow resulting in delay of normal erythropoiesis and in PRCA. The passive transfer of recipient’s type isoagglutinins by repeated RBC transfusions may also play the role in delayed onset of donor-type erythropoiesis.35

In conclusion, low level of isoagglutinin titters directed against donor’s blood antigens do not exclude the development of PRCA after ABO mismatched HSCT and the high effectiveness of anti-CD20 antibody confirms its immune-mediated patomechanism. In addition, the response to rituximab administered in lower doses, may reduce the cost.

PRCA followed by major ABO–incompatible allogenic HSCT usually recovers spontaneously.27 Nevertheless, the treatment may be necessary to avoid RBC transfusions and to decrease the risk of haemochromatosis. Some patients with PRCA after ABO mismatched HSCT, who become resistant to conventional treatment options, can benefit from rituximab therapy as it was proved in our study, but every one case should be individualized.
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