## Severe hematological side effects following Rituximab therapy in children

**Rituximab use in severe auto-immune diseases has recently increased. Scattered reports of opportunistic infections were the only reported serious side effects related to rituximab in pediatric patients .Here, we report transient severe acute thrombocytopenia and neutropenia respectively a few days after rituximab infusion in two children with autoimmune haemolytic anaemia. In both cases, cytopenia was reversible in a few days. The occurrence of cytopenias shortly after an infusion and their rapid reversibility suggest that these hematological side-effects were attributable to a direct toxicity of rituximab. Blood cell count must be carefully monitored after rituximab infusion in young children with autoimmune diseases.**

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The chimeric anti-CD20 monoclonal antibody rituximab is increasingly being used for treating severe autoimmune diseases. Its tolerance is generally good in adults as well as in children in such diseases. Scattered reports of opportunistic infections, including Pneumocystis carinii and varicella pneumonia, and enteroviral meningoencephalitis are the only reported serious side effects related to rituximab in pediatric patients,[1] and especially happen when multiple other immunosuppressive treatments have been used along with rituximab. Here, we report the occurrence of severe cytopenia following rituximab therapy in two patients with severe autoimmune hemolytic anemia (AIHA).

Case 1. A two-year old boy has been diagnosed as having an autoimmune lymphoproliferative syndrome (ALPS) type 1 presenting with an autoimmune hemolytic anemia (AIHA), splenomegaly and lymphadenopathy at age 3 months. Fas apoptosis deficiency was evidenced in this patient by the demonstration of an increased number of CD4 CD8 TCR $\alpha$,+ T cells, a decreased apoptotic response of activated T lymphocytes to anti-Apo 1-3 monoclonal antibody and the presence of a heterozygous mutation of the Fas receptor gene (exon 8). The patient was treated for severe AIHA from 6 to 24 months of age with steroid therapy, in combination with one course of ciclosporin. Because only a partial remission of AIHA was obtained under high dose of steroid therapy, rituximab was started. Before its administration, the haemoglobin level was 74 g/L, WBC count $6\times10^9$/L, neutrophil count $2.6\times10^9$/L and platelet count $192\times10^9$/L. Four weekly doses of rituximab at a dosage of 375 mg/m$^2$ were administered in association with steroid therapy at a dosage 2 mg/kg/day and were initially well tolerated. However, one week after the fourth rituximab infusion, the patient was admitted to hospital with a pyrexia of 41.5°C. Physical examination showed oral and anal ulcerations, and previously known hepatosplenomegaly and lymphadenopathy. His laboratory tests were as follow : WBC $1.9\times10^9$/L with neutrophils $0.02\times10^9$/L, haemoglobin 74 g/L and platelets $232\times10^9$/L. Chest X-ray was normal and blood cultures were sterile. Bone marrow smear analysis showed an increased cellularity associated with a maturation arrest occurring at the promyelocyte/myelocyte stage. There were no granulocyte-specific antibodies. Daily subcutaneous injections of G-CSF (granulocyte-colony stimulating factor), associated with one packed RBC transfusion and broad spectrum antibiotics for suspected bacterial infection (Vancomycin, Amikacin, Ceftazidime) were administered. Fever and mucous lesions resolved within a few days. G-CSF was stopped after three days, and his neutrophil count spontaneously increased to $1.5\times10^9$/L nine days after the onset of fever. Neutropenia has never relapsed after a 15 months follow-up. Platelet count remained also normal, but the AIHA did not improved.

Figure 1. Occurrence of severe neutropenia (patient 1) (A) and severe thrombocytopenia (patient 2) (B) after treatment with rituximab

Case 2. A three-year old boy presented with acute anaemia. Physical examination showed pallor and splenomegaly (3 cm below the costal margin). Laboratory tests revealed haemolytic anaemia with haemoglobin levels of 31 g/L and reticulocyte count $210\times10^9$/L, white blood cell count (WBC) $15\times10^9$/L, and platelet count 341 $\times10^9$/L. Warm-reactive autoantibodies of IgG type were demonstrated by direct antiglobulin test (DAT). Treatment with intravenous methylprednisolone was started at a dosage of 2 mg/kg/day. The patient required daily transfusion of packed red blood cells (RBC). Three pulses of 20 mg/kg methylprednisolone were given on consecutive days with no effect. Rituximab was started three weeks after the onset of anaemia, after 15 packed RBC transfusions have been received. It was administered intravenously at a dosage of 375 mg/m$^2$/dose, in association with concomitant steroid therapy at a dosage 2 mg/kg/day. Three weekly infusions of rituximab were well tolerated. Seven days after the third infusion, a repeated platelet count dropped from $165\times10^9$/L to $74\times10^9$/L, which worsened during the following two days to 22 x109/L (Figure). Epistaxis occurred and he required one platelet transfusion of 10 mls/kg. Post transfusion platelet count was $55\times10^9$/L. Bone marrow megakaryocytes were increased. His platelet count spontaneously increased to $190\times10^9$/L after 1 week and to $173\times10^9$/L after 2 months. Platelet and white blood cell counts remained normal, but the AIHA did not improved, with

five month follow-up.

According to the National Cancer Institute (NCI) Common Toxicity Criteria, neutropenia and thrombocytopenia with a severity grade 3 or 4 occurred respectively in 4.2 % and 1.7% of the patients treated for lymphoma.[2] Episodes of neutropenia have been observed either during the administration of rituximab, or with a delayed-onset after the completion of rituximab treatment in patients treated for B-lymphocytic malignancies.[3,4,5] Delayed-onset neutropenia occurred 4 to 42 weeks after the last administration of rituximab, and recovered within 4 to 148 days from onset; the degree of neutropenia ranged from nadir values of $0\times10^9$/L to $0.40\times10^9$/L. Severe transient acute thrombocytopenia was reported only in five patients,[6] a few hours after the first infusion of rituximab used either as a single agent or combined with chemotherapy mostly associated with cytokine release syndrome. A direct toxic effect of rituximab is unlikely, since granulocytes, platelets and haematopoietic precursor stem cells do not express CD20. The occurrence of neutropenia during the treatment, was attributed to the accelerated destruction of neutrophils caused by binding of rituximab-antigen complexes to neutrophil Fc receptors;[3] the same mechanism may be hypothesized for explaining the occurrence of thrombocytopenia. On the other hand, the development of delayed-onset neutropenia was possibly an immune-mediated neutropenia, secondary to a transient production of auto-antibody, during the acquisition of a new immune repertoire.[3] However, such haematologic toxicity has been rarely reported in patients treated for systemic autoimmune diseases.[7] In our patients, we believe that rituximab was responsible for the hematologic side effects observed in our patients since neutropenia and thrombocytopenia were transient, occurred a few days after an infusion of rituximab, and never relapsed after its completion. The occurrence of cytopenias shortly after an infusion, their rapid reversibility and the absence of anti-neutrophil antibodies in patient 2 suggest that these haematological side-effects were attributable to a direct toxicity of rituximab, rather than to autoimmunity. It has been recently reported that the rate of 5-10% of serum sickness in children treated for immune thrombocytopenic purpura was higher than in adults with lymphoma.[8] Thus, it cannot be excluded that children treated for auto-immune diseases presented with more side effects than adults with lymphoma after rituximab. The explanation for this higher rate of side-effects is unknown. We suggest that haematological testing should be performed at least once within the week following a rituximab infusion.

Rituximab is usually well tolerated. Nevertheless, these case reports illustrate that its use may be associated with severe haematological side effects in children, and that patients' full blood cell counts must be carefully monitored after rituximab infusions.

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