Pure red cell aplasia and anti-erythropoietin antibodies in patients treated with epoetin

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Abstract
Recombinant human erythropoietin (epoetin) was first used for the treatment of renal anaemia in 1986. During the first 10 years of its use, epoetin-induced antibodies were a rare complication and only three cases of patients with epoetin-induced antibodies associated with pure red cell aplasia (PRCA) were published. Since 1998, however, there has been a significant increase in the number of patients developing severe anaemia during the course of epoetin treatment due to neutralizing antibodies. Patients with PRCA present with an absolute resistance to epoetin therapy and then rapidly develop severe anaemia with a very low reticulocyte count (<10 000/mm³). Consequently, patients become dependent on blood transfusions to maintain an acceptable level of haemoglobin. By December 2002, approximately 142 patients worldwide had been diagnosed with antibody-positive PRCA after receiving epoetin. The vast majority of these patients had been treated with the Eprex™/Erypo™ brand of epoetin alfa, but there were also some cases in which patients had been receiving epoetin beta (Neorecormon™). To date, there have been no cases of antibody-mediated PRCA reported with the sole use of darbepoetin alfa (Aranesp®). All patients with epoetin-induced anti-erythropoietin antibodies had received the drug subcutaneously (s.c.), and almost all had chronic kidney disease-related anaemia. To our knowledge, no patient treated exclusively by intravenous (i.v.) administration has developed anti-erythropoietin antibodies. The increase in reported cases coincides with the removal of human serum albumin from the ex-US formulation of epoetin alfa, in order to comply with new regulations from the European regulatory authorities. It has been proposed that the new formulation is less stable, allowing aggregates of erythropoietin molecules to form, which increases the probability of antibody formation. Treatment with epoetin must be discontinued if PRCA is suspected. Patients do not respond to an increase in dose. Furthermore, patients must not be switched to another form of erythropoietic therapy as the antibodies cross-react with all erythropoietic therapies available. In around 70% of cases, immunosuppressive regimens are effective in eliminating the antibodies; cessation of epoetin therapy without concomitant immunosuppression is rarely effective. Kidney transplantation seems to provide an immediate and effective cure.

Keywords: anaemia; anti-erythropoietin antibodies; chronic kidney disease; pure red cell aplasia

Introduction

Pure red cell aplasia (PRCA) is a rare haematological disorder, which is defined by the absence of erythroblasts in the bone marrow [1]. The platelet and leukocyte counts remain normal in this condition, but erythroblasts make up <5% of the total cell count in the bone marrow. The erythroblasts remain in the early stages of development and do not mature. The result is profound anaemia, which is characterized by a very low reticulocyte count of <10 000/mm³.

Once PRCA has developed, the production of erythrocytes effectively ceases. The haemoglobin (Hb) concentration falls rapidly by ~0.1 g/dl/day, equating to 3 g/dl/month. This rapid fall in Hb concentration allows for an estimation of the time of erythrocyte production arrest. Serum iron and ferritin levels rise sharply as serum iron cannot be incorporated into the erythrocytes. Patients require repeated erythrocyte transfusions of up to 4 U of packed red cells per month. In PRCA not related to antibodies to erythropoietin, the levels of serum erythropoietin increase as a result of the reduced consumption of erythropoietin on erythroblasts. In PRCA caused by neutralizing antibodies...
to erythropoietin, however, serum erythropoietin levels determined by enzyme-linked immunosorbent assay (ELISA) are usually extremely low.

In adults, acquired PRCA can arise in association with thymoma, lymphoproliferative syndromes or an autoimmune-related disorder such as systemic lupus, rheumatoid arthritis or autoimmune hepatitis. It can also arise secondary to viral infections such as B19 parvovirus. Around half of the cases of PRCA in adults, however, are idiopathic in nature where no aetiology can be established.

Idiopathic PRCA is usually an autoimmune disorder in which immunoglobulin G (IgG) antibodies or cytotoxic T lymphocytes are directed against erythroid progenitors or precursors, although in rare cases, antibodies against endogenous erythropoietin have been reported in patients who have never received erythropoietic agents. Over the past 20 years, only four such reports have been published [2–5]. From 1988 (when epoetin was first commercially available) to 1999, only three fully documented cases of epoetin-associated PRCA had been reported — these reports came from Germany, Spain and the USA [6–8]. A recent increase in the number of cases of PRCA associated with epoetin treatment has prompted increased interest in this previously rare syndrome.

**Evidence for anti-erythropoietin antibodies**

Immunoassays are most often used to test for the presence of anti-erythropoietin antibodies. One such assay, an immunoprecipitation test, examines the capacity of serum to precipitate iodinated epoetin in the presence of Protein G-Sepharose. Increasing concentrations of patient serum are incubated with $^{125}$I-epoetin and immune complexes are separated out using agarose-bound Protein G. Figure 1 shows the result for one patient [9]. Approximately 1 ml of the patient’s serum was able to precipitate 40 IU of epoetin. The quantity of serum correlated positively with the amount of immunoprecipitated epoetin. Control serum did not cause any epoetin precipitation. This test confirms the presence of anti-erythropoietin antibodies in the patient sera, but it does not prove that the antibodies are neutralizing.

Two methods are used to detect neutralizing antibodies. In the first method, the effect of the patient’s serum on the growth of erythroid progenitor cells is tested using erythroid progenitors obtained from healthy donors. Figure 2 shows the results for the same patient as in Figure 1. The patient’s serum fully inhibited the growth of erythroid progenitor cells stimulated with 1 IU/ml epoetin. Increasing the concentration of epoetin in the culture reverses this inhibition, however, and the inhibitory effect was no longer observed in the presence of 50 or 100 IU/ml epoetin. Moreover, when the patient’s serum was depleted of IgG, the growth of erythroid progenitor cells was restored with just 1 U of epoetin. The purified IgG inhibited erythroid growth in the presence of the same concentration of epoetin (1 IU/ml). Patient sera did not modify the formation of granulocytic colonies stimulated by granulocyte colony-stimulating factor (G-CSF), showing that the IgG present in the patient sera is specific to erythroblastic progenitors. This technique is highly specific, but is time consuming and difficult to perform, so a second method is being increasingly used, involving the human UT-7 erythroleukaemia cell line. Proliferation of this cell line is dependent on the presence of either epoetin or granulocyte–macrophage colony-stimulating factor (GM-CSF). Control serum and sufficient epoetin allowed growth of the cell line, but patient sera completely inhibited epoetin-stimulated proliferation of UT-7 cells (Figure 3). This inhibition was again reversed at higher concentrations of epoetin. Although this second test is more practical because it uses a human cell line, UT-7 cells are sensitive to other growth factors that may be present in the patient sera. These two tests can, however, provide strong evidence for the presence of neutralizing anti-erythropoietin antibodies in patient sera.

In PRCA, serum erythropoietin levels are usually very high. In contrast, in patients with antibody-associated PRCA, the serum concentrations of erythropoietin detected with an ELISA are extremely low.
Characterization of anti-erythropoietin antibodies

Epoetin molecules are heavily glycosylated and the glycosylation is slightly different from that of the endogenous erythropoietin molecule [10,11]. We therefore investigated whether the antibodies were directed against the carbohydrate residues or the protein core. Using an immunoprecipitation assay, anti-erythropoietin antibodies were shown to bind to both the native glycosylated and the deglycosylated forms of erythropoietin [12] (Figure 4). The antibodies recognized the core protein of endogenous erythropoietin. The high-affinity antibodies did not bind denatured erythropoietin, suggesting that these antibodies are directed against a conformational epitope of the protein moiety and not to the glycosylation [13]. In each case, the level of immunoprecipitating antibodies was able to neutralize very large amounts of erythropoietin with an affinity similar to that of the erythropoietin receptor. These antibodies are therefore able to neutralize all erythropoietin molecules and fully inhibit erythropoiesis. The antibodies varied in their binding capacities, however, from 1.5 to 277 U/ml at diagnosis. Normal serum erythropoietin concentrations are ~20 mU/ml. The binding capacity of the antibodies can easily overcome this concentration of erythropoietin. This high binding capacity explains why increasing the therapeutic dose of epoetin in patients is ineffective.

Increase in cases of PRCA with neutralizing anti-erythropoietin antibodies

Epoetin has been used successfully for many years to correct the anaemia of chronic renal failure without significant reports of anti-erythropoietin antibodies. The number of cases in the past 4 years, however, has increased sharply. Globally, between 1998 and March 2003, there have been 175 antibody-positive cases of PRCA in renal patients receiving s.c. epoetin. Of these cases, 142 patients had been treated with the EprexTM brand of epoetin alfa only, eight had received epoetin beta only, and 21 had been exposed to both epoetin alfa and epoetin beta [14].

Careful examination of the patients’ history showed no correlation between the development of PRCA and underlying renal disease or human leukocyte antigen. There have been no changes in medical practice or introduction of new drugs since 1998 that can explain the increase in cases. In addition, the possibility of a change in the dialysis membrane producing changes in the epoetin molecule, rendering it more immunogenic, can be excluded because some patients were not receiving haemodialysis. It has also been considered that small amounts of silicone, used to lubricate pre-filled syringes, may enhance the antigenicity. In 1998, however, there was a change in the European epoetin alfa (EprexTM/ErypoTM) formulation. Human serum albumin (HSA), which acts as a stabilizer, was removed to comply with new European regulations and replaced with synthetic polysorbate 80. It is possible that the removal of HSA may have decreased the stability of the molecule and increased its immunogenicity, especially when administered by the s.c. route. The increased incidence of PRCA coincides with the introduction of HSA-free EprexTM/ErypoTM, although the evidence remains circumstantial. In the USA, the use of HSA as a stabilizer is still permitted and the formulation of epoetin alfa has not been changed. No increase in the number of cases of PRCA with neutralizing anti-erythropoietin antibodies has been reported in the USA. It should be noted that the formulation of epoetin beta has always been HSA-free, but the stabilizer composition differs from that of epoetin alfa. There are subtle differences between the carbohydrate moieties of epoetin alfa and epoetin beta.
but it is not known whether these have any impact on immunogenicity. Immunoprecipitation assays have shown that the anti-erythropoietin antibodies in patients with PRCA are directed against the protein moiety of the molecule [9].

**History and time course of individual PRCA cases**

**Typical case study**

In 1998, a 72-year-old man with vascular nephropathy was referred to us with PRCA. He was on maintenance haemodialysis and had been started on epoetin alfa (Eprex™) 4000 IU/week for the treatment of renal anaemia. This dose had a mild effect and consequently, after 6 months, the dosage was increased to 6000 IU/week, and his Hb concentration increased from 10 to 12 g/dl. After 6 months, however, the patient suddenly developed severe anaemia. His Hb concentration decreased by 3 g/dl in 1 month, and at a Hb concentration of 6 g/dl, his reticulocyte count was extremely low at 7000/mm³. His serum ferritin levels had increased sharply after the development of anaemia. This patient met the clinical criteria for possible PRCA and the diagnosis was confirmed by bone marrow examination. The patient had not required transfusion during treatment with epoetin alfa, but now needed to be heavily transfused with ~6 U of packed erythrocytes per month.

**Experience at the Hôpital Hôtel-Dieu**

Since 1998, we have tested sera from more than 80 patients for anti-erythropoietin antibodies at our laboratories. Two of the samples were from patients with myelodysplastic syndrome, and the rest were from renal patients who had been treated with epoetin. Antibodies were detected only in those patients who had received epoetin therapy. Patient sera originated from Europe, Australia and Canada, with France presenting the most cases, closely followed by the UK. For 31 patients, the bone marrow results were not available, but in 48 patients, the diagnosis of PRCA was confirmed. All the clinical histories in these patients were very similar.

Results from these 48 patients are summarized in Figure 5. Many patients received epoetin alfa (Eprex™) only by the s.c. route when they developed anaemia. Two patients had received epoetin beta (NeoRecormon™) by the s.c. route. Some patients had received more than one epoetin type. Only one patient diagnosed with PRCA had received darbepoetin alfa; this patient, however, had been treated previously with epoetin alfa and beta. Examination of stored serum from this patient established the presence of anti-erythropoietin antibodies before the start of darbepoetin alfa therapy.

The mean time between starting epoetin therapy and the onset of anaemia in this series was 9 months (range, 2–63 months). It can be deduced that the development of anti-erythropoietin antibodies is secondary to treatment with epoetin. PRCA was clearly linked to the presence of anti-erythropoietin antibodies; cryopreserved sera from five patients before the development of PRCA were negative for anti-erythropoietin antibodies. However, the time course from initiation of immune response to the development of antibody-mediated PRCA (the immunization process) remains unknown. In addition, because antibodies cross-react with all epoetins, switching products may not stop the progression to PRCA.

**Practical approach to treatment and follow-up**

A reticulocyte count should be performed in any patient whose Hb levels rapidly decrease during epoetin treatment. Patients receiving epoetin therapy should have reticulocyte counts of between 30,000 and 200,000/mm³ (mean, 80,000/mm³). A count of

![Fig. 5. Time course of individual PRCA cases after erythropoietic therapy.](https://academic.oup.com/ndt/article-abstract/18/suppl_8/viii137/1823672/Pure-red-cell-aplasia-and-anti-erythropoietin)
PRCA and anti-erythropoietin antibodies in epoetin-treated patients 10 000/mm$^3$ or less in a patient with rapidly increasing anaemia is indicative of PRCA, but other potential causes of anaemia such as severe iron deficiency must be eliminated. If PRCA is strongly suspected and no other cause has been identified, a bone marrow examination should take place and the patient’s serum should be investigated for anti-erythropoietin antibodies. If the diagnosis of PRCA with antibodies to erythropoietin is confirmed, then epoetin treatment must be discontinued immediately. Increasing the dose of epoetin is not effective and the patient should not be switched to another form of erythropoietic therapy as the anti-erythropoietin antibodies can cross-react with all other forms of erythropoietic therapy.

Forty-two patients have been followed up for their response to treatment. Of those patients, 33 were French, three were German, three were from the UK and three were from other European countries. They had all been treated with epoetin alfa for anaemia of chronic kidney disease and had subsequently developed PRCA between June 1998 and December 2002. Six of the patients were not receiving dialysis. Thirty-three patients were followed for 1 year. Recovery was defined as the disappearance of antibodies and a reticulocyte count of $>10 000$/mm$^3$. Four patients received no treatment for PRCA and have not recovered, but of the 29 patients who did receive treatment, 72% recovered. Various approaches to treatment have been tried, with some patients receiving more than one treatment. The best results to date have been seen with the combined use of corticosteroid and cyclophosphamide. The best treatment overall, where possible, is kidney transplantation because the antibodies do not return. Patients were not re-challenged with epoetin therapy, so it remains uncertain whether treatment with epoetin (i.v.) can be resumed after the disappearance of antibodies.

Conclusions

Since 1998, there has been a sharp increase in the incidence of epoetin-induced PRCA with neutralizing anti-erythropoietin antibodies outside the USA. The most probable cause appears to be a change in the European formulation of the Eprex$^\text{TM}$/Eryo$^\text{TM}$ brand of epoetin alfa, in which HSA has been replaced by synthetic polysorbate 80. Patients with PRCA become dependent on transfusions until renal transplantation or effective treatment can be offered. Treatment with immunosuppressive agents has been effective in some patients; although the number of these cases is small, the best results to date have been achieved with a combination of corticosteroids and cyclophosphamide.

References