Recruiment Human EPO has revolutionized the management of renal anemia with improvement in outcomes and quality of life. Its side effects are mild and limited. Though EPO is considered relatively non-immunogenic (1), several cases have been reported in the literature where patients have developed EPO antibodies as a consequence of treatment with EPO, failed to respond to EPO and developed pure red cell aplasia (PRCA) (2). Over the last few years, however, there has been an increase in the number of patients developing neutralizing anti-EPO antibodies and PRCA; over 165 cases have been reported worldwide (3). In this syndrome the patients become blood transfusion dependent and generally therapy with other EPO preparations is not advised, because of cross-reaction with EPO antibodies and potential allergic skin and systemic reactions (4). Cessation of EPO alone is usually insufficient to induce recovery. Administration of immunosuppressive therapy appears greatly to enhance the likelihood of recovery (5) and is completely cured by renal transplantation (6). Whether EPO therapy can be safely resumed at some stage is unclear, and remains a crucial question for patients who have recovered from EPO-induced PRCA.

We report here a patient on peritoneal dialysis (PD) who developed EPO antibodies and PRCA and became blood transfusion dependent. He subsequently did respond to Darbepoetin alpha (Aranesp), in spite of persisting EPO antibodies. We believe that this is a first report where a patient responded to another EPO preparation despite developing EPO antibodies and PRCA.

**Case Reports**

An 82 yr old retired medical practitioner, presented in end stage renal failure from renovascular disease in 1997. He had significant ischemic heart disease with previous myocardial infarction and coronary artery by-pass surgery (1992). He also had generalized arteriopathic disease along with hypertension. Soon after presentation he was commenced on continuous ambulatory peritoneal dialysis (CAPD). He tolerated this well with improvement in his biochemistry and uremic symptoms. Renal anemia persisted following CAPD, and he was commenced on subcutaneous Epoetin alpha (Eprex) 3000 units twice weekly. His pre-EPO hemoglobin (Hb) was 8 g/dl. He responded well to this, with an increase of his hemoglobin to 14.2 g/dl by September 1998. His Hb started declining by May 1999, and 3 mo later had dropped to 11.5 g/dl. Despite increasing EPO dose, his Hb continued to decline and he became severely anemic (Hb 6.6 g/dl) and transfusion dependent. (Table 1 and 2).

Investigations to ascertain a cause for the anemia were essentially negative. He had no evidence of infection, malignancy, hyperparathyroidism or gastrointestinal bleeding, hemolysis or iron deficiency. His serum B12, serum folate and serum ferritin levels were essentially normal being 483 ng/l, 8.3 ng/l and 191.3 ng/l respectively. His PTH level was 90 pg/ml. His serum was tested for parvovirus IgM antibody and was negative. A bone marrow aspiration in December 1999 showed absent erythroid activity with no evidence of giant erythroblasts and normal white cell and platelet count, consistent with clinical picture of persistent anemia and pure red cell...
aplasia. Cytogenetic analysis of bone marrow showed normal male karyotype. No clonal abnormalities were detected. Neutralizing antibodies to EPO (November 1999) were positive (by radio-immunoprecipitation assay). Unfortunately it took an inordinate long time for the EPO antibody positive report to ‘filter’ back; hence the patient remained on EPO for an inappropriately long time. As soon as EPO positive report was available, epoetin alpha was withdrawn. The patient remained blood transfusion dependent, requiring on the average two units of blood weekly (Table 2) and maintain him asymptomatic of angina and heart failure. No serial or simultaneous erythropoietin levels were measured.

In November 2001, the patient was commenced on Darbepoetin alpha (Aranesp) as the repeated need for blood transfusion was impacting on his quality of life; in addition his serum ferritin level had now exceeded 5000 ng/L. The patient was desperate for any alternative to blood transfusion therapy, which was becoming intolerable. In spite of a detailed explanation of cross-reaction of the EPO antibodies with Darbepoetin alpha, with the potential of systemic reaction, he undertook the therapy and the risk; being a medical practitioner, he fully understood the basis of the risk.

Darbepoetin was commenced in November 2001, with a dose of 40 microgram once weekly, administered subcutaneously. He showed a dramatic response in his Hb levels, which increased and reached a peak level of 15.2 g/dl in July 2002 (Table 3, Figure 1); no blood transfusion were required after December 2001. With dose reduction, he maintained an Hb level of around 12–13 g/dl at a dose of 30 micrograms weekly.

This patient died of myocardial infarction on 14 November 2002 at home. The last hemoglobin level as an outpatient was 13.1 g/dl.

Antibodies to erythropoietin
Erythropoietin antibody testing was undertaken using the radioimmunoprecipitation assay (RIP) performed in the laboratories of Ortho-Biotech (manufacturers of Eprex - epoetin alpha) and also independently at the Department of immunology, Guy’s, King’s and St Thomas’ School of Medicine, University of London. The technique involves serum samples being incubated first with 125I-radiolabeled epoetin. Subsequently serum IgG antibodies are precipitated with Protein G agarose. The presence of radioactivity in the resulting pellet indicates anti-EPO antibodies. The results showed that 5 micro liters of serum bound 33.7% of iodinated epoetin and 2 micro liters of serum bound 12.1% of iodinated epoetin. The RIP assay cannot distinguish neutralizing from non-neutralizing antibodies. However, binding in the region of 30% or above is thought to be invariably accompanied by neutralizing activity.

Subsequently the antibodies were retrospectively assayed in the laboratories of Amgen Clinical Immunology Department in USA (manufacturers of Aranesp Epogen) and using the bio-

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<td>—</td>
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Table 3. Aranesp dose and haemoglobin response in spite of persistence of EPO antibodies and without the need of blood transfusions
sensor immunoassay (BIAcore 3000) to determine whether the serum from this patient contained, in addition, anti-drug antibodies. This assay uses a biosensor instrument. The patient specimen is passed over a biosensor surface with drug covalently immobilized (to its surface). Antibodies are indicated by an accumulation of mass and subsequent confirmation with an anti-Ig reagent on the biosensor. This assay showed evidence of anti-drug antibodies. Assay of his neutralizing antibody titer during the period of therapy with Darbepoetin alpha showed their continued presence (MRIA binding 38%). Table 4 shows the presence of antibodies to different EPO preparations. Anti-EPO antibodies were declining over time, especially during the Darbepoetin treatment phase.

The specimens from this patient were positive for antibodies against Eprex. The specimens were further characterized but no antibody isotype was identified due to the low concentration of antibody present. Cross reactivity was observed against epoetin alpha (Epogen), epoetin beta (Neorecormon), and Darbepoetin alpha (Aranesp).

**Discussion**

We report here a case of a patient on PD, who developed EPO antibodies and PRCA, subsequently becoming EPO resistant and blood transfusions dependent. This patient responded well to Darbepoetin alpha, in spite of persisting antibodies. This, we believe is a new and interesting observation, not so far reported.

The pathophysiology of PRCA is heterogeneous (3). In the area of chronic renal failure and dialysis, PRCA has been almost entirely related to patients receiving EPO, predominantly via the subcutaneous route (2). Over 165 cases of confirmed or suspected PRCA have been reported in chronic renal failure patients treated with epoetin alpha, mostly occurring after 1998 (1,3,5). The general advice that chronic renal failure patients developing EPO antibodies and PRCA should not be switched to another erythropoietin is related to the presumed assumption that EPO antibodies will certainly cross-react with other EPO preparations (3,5) and there is the risk of severe systemic immune reactions (4).

This patient responded to Darbepoetin alpha in spite of the presence of EPO antibodies, which were nevertheless, declining. He did not suffer from any systemic reactions as was so in the report by Weber et al. (4). It is interesting to contemplate the reasons for this success course. In situations where a recombinant product is used to substitute for deficient production of an endogenously produced molecule, antibody formation represents a break down of self-tolerance. Such an immunogenic reaction can be triggered by a variety of factors including variation in protein sequence, differences in glycosylation, protein aggregation, contaminants, and impurities occurring during the production process (8). In addition factors such as individual predisposition, and patient immune status may be of importance. In this respect it is conceivable that, while the anti EPO antibodies were present and demonstrated against all the molecules, the differences in glycosylation of the Darbepoetin molecule from that of epoetin alpha and beta may have abrogated any negative impact on the erythropoietic action of Darbepoetin. It is conceivable that slight differences in glycosylation pattern or secondary changes induced by formulation lead to the presentation of previously hidden epitopes or generation of structures that are immunogenic; its clearly possible that this was not so for Darbepoetin in our case and likely to be so in the Weber report. The carbohydrate moiety of Darbepoetin alpha is vastly different from those of the other EPO preparations (9). Furthermore, the antibodies levels were in decline and may have modified the response against Darbepoetin alpha. It is of interest that our case did not react to the Darbepoetin as compared to the case repost of Weber (4). This is difficult to surmise but may be down patient specific factors.

![Graphical representation of hemoglobin (Hb) levels (gm/dl) during the 3 phases in the dialysis lifespan of this patient.](image)

**Figure 1.** Graphical representation of hemoglobin (Hb) levels (gm/dl) during the 3 phases in the dialysis lifespan of this patient. The first phase (EPO starts good response) entailed treatment with epoetin alpha. The second phase was the period of nearly 2 yr when he was transfusion-dependent. Finally, the third phase showed the good response to Darbepoetin alpha (Aranesp).

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**Table 4.** The levels of anti-EPO antibodies from patient’s serum to different EPO preparations
In our case, the epoetin alpha was continued well after the antibodies became positive; it is possible that our patient was less ‘immunogenic’ in spite of the antibodies, to evoke a systemic response as in the case of Weber. Finally, EPO antibodies can be self-limiting with spontaneous remissions, but in our case, presence of EPO antibodies was demonstrated even after two years of stopping epoetin alpha, although the levels were declining. Whether this had an impact on the positive response is difficult to surmise.

In our case, no immunosuppressive treatment was used. Cyclosporine has been used to treat this disorder in dialysis patients (5,6).

This is a new observation. In spite of the generally held view of ‘not challenging patients with EPO antibodies and PRCA with another erythropoietin protein’ (7), since antibodies cross-reacted with all commercially available recombinant erythropoietic products, we believe that in patients as ours, who are desperate, a trial of Darbepoetin is worthwhile after ‘informed consent’ is obtained. We believe that more research and information is required to understand the highlighted issue in this case report.

Acknowledgements
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References