Characteristic Changes in Anti-erythropoietin Antibodies in a Peritoneal Dialysis Patient Who Developed Pure Red Cell Aplasia

Shu-Hong Bi,1,2 Li-Tao Cheng,1 Wei Chen,1,2 Tao Wang1,2

Although cases of anti-erythropoietin (anti-EPO) antibody-induced pure red cell aplasia (PRCA) have been reported since 1998, the role of anti-EPO antibodies in the development of PRCA is controversial, with many conflicting opinions. Here, we present a patient who had serial serum anti-EPO antibody levels measured both before and after the development of PRCA to observe the relationship between serial changes in anti-EPO antibody and hemoglobin levels. Enzyme-linked immunosorbent assays were used for the detection of anti-EPO antibodies. The patient’s serum anti-EPO antibody levels showed a characteristic change over time: negative before the development of PRCA, strongly positive when PRCA was diagnosed, decreasing after receiving prednisone, and eventually became negative. More importantly, an inversely close association between changes in serum anti-EPO antibody and hemoglobin levels was shown. The characteristic changes in this case indicate that anti-EPO antibody might have a role in the development of PRCA, and monitoring the titer of anti-EPO antibody may be of great value in the diagnosis and treatment of PRCA. [Hong Kong J Nephrol 2006;8(2):71–4]

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CASE REPORT

A 77-year-old male was admitted to hospital in August 2003 with the complaints of weakness, easy fatigability and bad appetite for 4 months. He was diagnosed with chronic renal failure and put on continuous ambulatory peritoneal dialysis. He tolerated it well and showed improvement in his biochemistry and uremic symptoms. However, as renal anemia persisted following continuous ambulatory peritoneal dialysis, epoetin alfa 3,000 U thrice weekly was administered subcutaneously. The patient recovered well and his hemoglobin level increased continuously even with tapered EPO doses. By February 2004, his hemoglobin had increased to and remained stable at 13.3 g/dL.

In April 2004, however, his hemoglobin level started to decline without any obvious cause and, 3 months later, had dropped to 4.4 g/dL. Although increased doses of epoetin were prescribed, his hemoglobin level continued to decline and he became severely anemic and transfusion-dependent (Figure). His hemoglobin was 4.4 g/dL, hematocrit was 12.6%, mean corpuscular volume was 93 fL (normal range, 80.2–97.7 fL), and absolute reticulocyte count was $3.8 \times 10^9/L (0.18\%); white blood cell, platelet and differential white cell counts were normal. Ilium marrow aspirate showed the absence of erythroblasts. Megakaryocytes and myeloid series were normal in morphology and maturation. Another bone marrow aspirate, but from the sternum, showed the same results. He had no evidence of infection, malignancy, hyperparathyroidism, gastrointestinal bleeding, hemolysis or iron deficiency, and serum levels of vitamin B12, folate and ferritin were normal. His parathyroid hormone level was 37 pg/mL (normal range, 9–55 pg/mL). Antinuclear antibody, antibody to double-stranded DNA, antibody to hepatitis C virus and hepatitis B virus surface antigens, antibody to HIV and rapid plasma reagin test were all negative. X-ray and computed tomography of the chest did not reveal thymoma. Bone marrow examination and the clinical picture strongly suggested PRCA. Therefore, tests for anti-EPO antibodies (from a fresh sample in June 2004 and a frozen serum sample from February 2004) were performed using enzyme-linked immunosorbent assays.

Briefly, the purified known EPO antigens were diluted to 2 µg/mL in coating buffer (0.05 M bicarbonate buffer, pH 9.6) and coated onto the wells of one half of a Costar microtiter plate (Costar Data Packaging Corp., Cambridge, MA, USA); the wells in the other half were coated with coating buffer and acted as antigen-free wells. The coated plates were incubated at 37°C for 1 hour, and then washed three times with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST) between stages. Test sera were

![Figure](https://via.placeholder.com/150)

**Figure.** There is an inverse association between change in anti-EPO antibody titer and change in hemoglobin level in this EPO-induced PRCA patient. The broken (dashed) line represents the standard, normal level of anti-EPO antibody (23%). The white columns represent anti-EPO antibody levels.
diluted 1:50 with PBST and coated in duplicate on both antigen-coated wells and antigen-free wells; every plate contained positive, negative and blank (PBST) controls. Volumes in the wells at this step and subsequent steps were all 100 μL. Binding was detected with horseradish peroxidase-conjugated anti-human IgG (100 μL at 1:2,000 dilution) for 30 minutes at 37°C. The peroxidase substrate o-phenylenediamine was used at 0.4 mg/dL in 0.1 M citrate buffer (pH 5.0). The reaction was stopped by 1 mol/L HCl. Results (reported in optical density, OD) were recorded as the net OD \text{\textsubscript{490nm}} (average value on antigen wells minus average value on antigen-free wells) and expressed as a percentage according to a linear regression equation established on the basis of different OD values from a negative control and a positive control (OD: 1%, 100% individually). For the control, serum samples from patients treated with epoetin who did not have PRCA were tested and an average value of 23% was found. Titer > 23% was defined as a positive result.

Our patient’s anti-EPO antibody as measured from the frozen serum sample taken in February 2004 turned out to be negative, but had changed to be strongly positive (117%) in June 2004 (Figure). After discussion between expert nephrologists and hematologists, the diagnosis of PRCA was made. The administration of EPO was stopped and a treatment course with oral prednisone was begun in July 2004 (1 mg/kg daily for 4 weeks, with subsequent tapering of the dose). Eight weeks after the onset of steroid therapy, the patient’s serum hemoglobin had stabilized at approximately 8 g/dL without further blood transfusions. His anti-EPO antibody titer had also decreased substantially to 33.5%, and absolute reticulocyte count had increased from 3.8 × 10\textsuperscript{9}/L (0.18%) to 61.32 × 10\textsuperscript{9}/L (2.8%). The patient continued to respond well to prednisone; by January 2005, hemoglobin had increased to 10.8 g/dL and detection of anti-EPO antibody was negative.

**DISCUSSION**

The major discovery from this case was the inverse relationship between the changes in anti-EPO antibody and hemoglobin levels in this EPO-induced PRCA patient. It is well known that all exogenous proteins, including therapeutic ones, are potentially immunogenic. Although rhHuEPO is identical to endogenous human EPO with regard to its physicochemical properties, it remains potentially immunogenic as it is an exogenous protein [2]. In a study by Casadevall et al, the identification of anti-EPO antibodies in 13 patients who had been treated with EPO and who developed PRCA thereafter suggested that this recombinant hormone may have played a role in the etiology of PRCA [3]. Anti-EPO antibodies can neutralize all EPO molecules *in vitro* [6], which is believed to be a major mechanism for inhibiting erythropoiesis in the body.

However, conflicting reports have emerged in the literature in recent years, which put the role of anti-EPO antibodies at the center of the controversy. Kharagjitsingh et al reported no definite relationship between anti-EPO antibody and PRCA in their study [4]. In their anti-EPO antibody screening of the sera of 1,403 dialysis patients on EPO, three patients were found to have developed anti-EPO antibodies. However, none of them had a diagnosis of EPO-induced PRCA and they were all negative for their neutralizing ability. There was only one PRCA patient found, who was negative for anti-EPO antibody [4]. This observation has been supported by other studies [5,7]. These discrepancies might be related to the different affinities of the antibodies. It is possible that a patient with a positive but low affinity anti-EPO antibody will have a negative neutralizing assay result.

Our patient was diagnosed with EPO-induced PRCA on the basis of clinical characteristics, bone marrow examinations and positive detection of anti-EPO antibody in serum. In addition, other possible causes of PRCA, such as infections, drugs, autoimmune disorders, and malignancy, had been excluded. The highlight of this case is that it presented a characteristic change in anti-EPO antibody titer and a concomitant change in serum hemoglobin level. A negative result for anti-EPO antibody was found in the initial period of epoetin alfa treatment and the patient responded well to EPO. After 10 months of EPO administration, however, anti-EPO antibody became strongly positive when the patient showed obvious EPO resistance and progressive decrease in serum hemoglobin. After the discontinuation of EPO and the onset of steroid therapy, anti-EPO antibody titers dramatically decreased and eventually became negative, followed by a gradual increase in serum hemoglobin. To the best of our knowledge, this is the first report to show longitudinal changes in anti-EPO antibody in the course of EPO-induced PRCA in a dialysis patient. Other than the inverse association between anti-EPO antibody titer and hemoglobin level, the start of steroid therapy was accompanied by a reversal of the trend of increasing anti-EPO antibody and a noticeable improvement in anaemia independent of transfusion, which further proved the key role of anti-EPO antibody in the pathogenesis of EPO-induced PRCA. The efficacy of the immunosuppressive therapy in this patient’s EPO-induced PRCA is also in agreement with previous studies [8–10]. The inverse relationship between the change in anti-EPO antibody titer and hemoglobin concentration in this study suggests that anti-EPO antibody plays a critical role in the pathogenesis of EPO-induced PRCA.
In conclusion, this case presented characteristic changes in serum anti-EPO antibody in a peritoneal dialysis patient receiving EPO who developed PRCA. The characteristic changes indicated that anti-EPO antibody plays a role in the development of PRCA, and monitoring the titer of anti-EPO antibody may be of great value in the diagnosis and treatment of PRCA.

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REFERENCES