Pure red cell aplasia due to follow-on epoetin

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CASE PRESENTATION

A 57-year-old man with end-stage renal disease secondary to chronic interstitial nephritis was initiated on hemodialysis in February 2006 for uremic symptoms. In April 2006, his hemoglobin (Hgb) was 10.7 g/dl and transferrin saturation was 12.7%. He was started on subcutaneous follow-on epoetin-alfa, Wepox (Wockhardt Limited India, Mumbai, India) and 10 weekly doses of 100 mg each of iron sucrose administered intravenously. His Hgb increased to 12.3 g/dl within 2 months after the initiation of epoetin. He then underwent coronary artery bypass graft surgery for triple vessel coronary artery disease. He received two units of packed cells during the post-operative period because of a decrease in the Hgb level to 8.2 g/dl. He was continued on epoetin at the same dose. Three months later the Hgb level later was 6.6 g/dl (Figure 1). Two units of red blood cell transfusion and intravenous iron were administered, and Wepox was continued. In December 2006, his Hgb decreased further to 4.9 g/dl. Further work up of his anemia revealed that a stool sample for occult blood was negative, an upper gastrointestinal endoscopy showed an antral ulcer, and gastric biopsy for *Helicobacter pylori* was positive. He was treated with ampicillin, tinidazole, and omeprazole for 10 days. A repeat endoscopy, 1 month later, revealed a healed duodenal ulcer. A peripheral smear showed reduced reticulocytes (0.8%) with normal platelet and myelocyte series. His serum transferrin saturation was 97.8%. lactate dehydrogenase was 385 U/l. Parvovirus B19 serology was negative (IgM level was 9.3 U/ml (negative if less than 17 U/ml)).

He received, on an average, 4 units of packed red cells per month. A bone marrow trephine (Figure 2a) and aspirate (Figure 2b) showed a selective depletion of erythroblast precursors with normal granulopoiesis and megakaryopoiesis suggestive of pure red cell aplasia (PRCA). Wepox was withheld. His serum erythropoietin level was 558 mU/ml (normal range 10–30 mU/ml). The serum anti-erythropoietin antibody assay was positive (0.9% c.p.m. at 1:100 dilution and 4.3% c.p.m. at 1:20 dilution by radioimmunoprecipitation assay (RIPA)).

CLINICAL DIAGNOSIS

Pure red cell aplasia due to Wepox.

CLINICAL FOLLOW-UP

After discontinuation of Wepox, he received 6 units of blood transfusion and azathioprine at a dose of 300 mg per day, after which his hemoglobin (Hgb) increased to 9.3 g/dl within 2 weeks. He then underwent live unrelated renal transplantation in January 2007, 2 weeks after stopping Wepox. Since then, he has been managed on triple drug immunosuppression comprising of tacrolimus, mycophenolate mofetil, and prednisolone. The post-transplant course has been notable for two episodes of acute rejection, which responded to methylprednisolone. After his kidney transplant, he is not anemic. His Hgb and serum creatinine at 5 months post-transplant were 12.7 g/dl and 0.9 mg/dl, respectively.

DISCUSSION

This presentation highlights the occurrence of PRCA with follow-on epoetins (FOEs). In Europe, FOEs are also referred to as biosimilar epoetins. In our patient, PRCA developed because of neutralizing IgG antibodies to the protein component of administered FOE, Wepox, that cross-reacted with endogenous epoetin. The precise mechanism for PRCA in patients treated with FOEs has not been elucidated. However, because PRCA from innovator epoetin appears to have resulted from problems in the manufacturing and storage of epoetin that have since been addressed, it is likely that this could also be the case with FOE, such as Wepox. Additionally, because FOEs are largely marketed in emerging countries where there is limited pharmacovigilance, surveillance for cases of FOEs-induced PRCA takes on even greater significance.

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Kidney International (2008) **74,** 1617–1622; doi:10.1038/ki.2008.230; published online 11 June 2008

Received 2 December 2007; revised 15 March 2008; accepted 26 March 2008; published online 11 June 2008

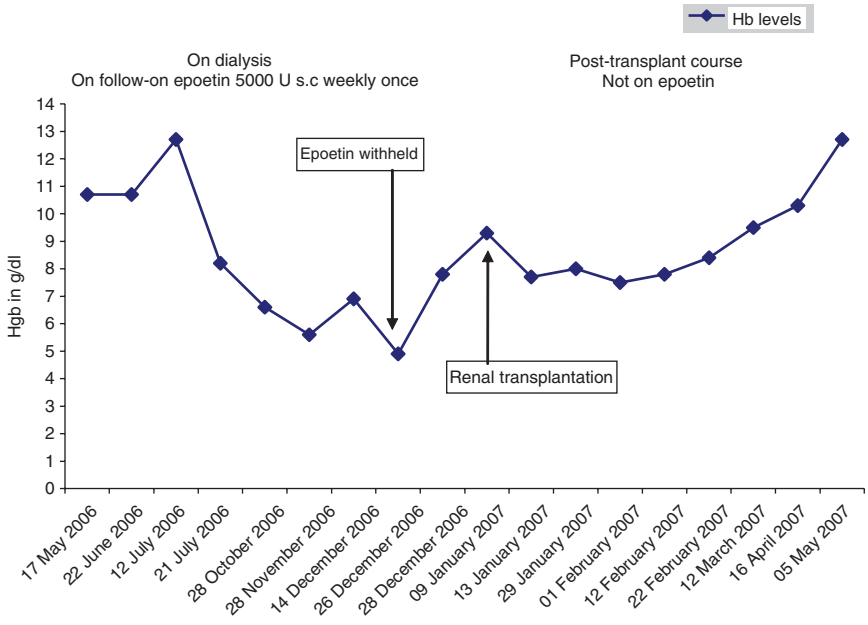

Figure 1 | Hgb level over time in the index case. Clinical course of the index patient with PRCA due to FOE showing schematic depiction of Hgb levels during the pre-transplant and post-transplant period, and the dose and route of administration of follow-on epoetin.

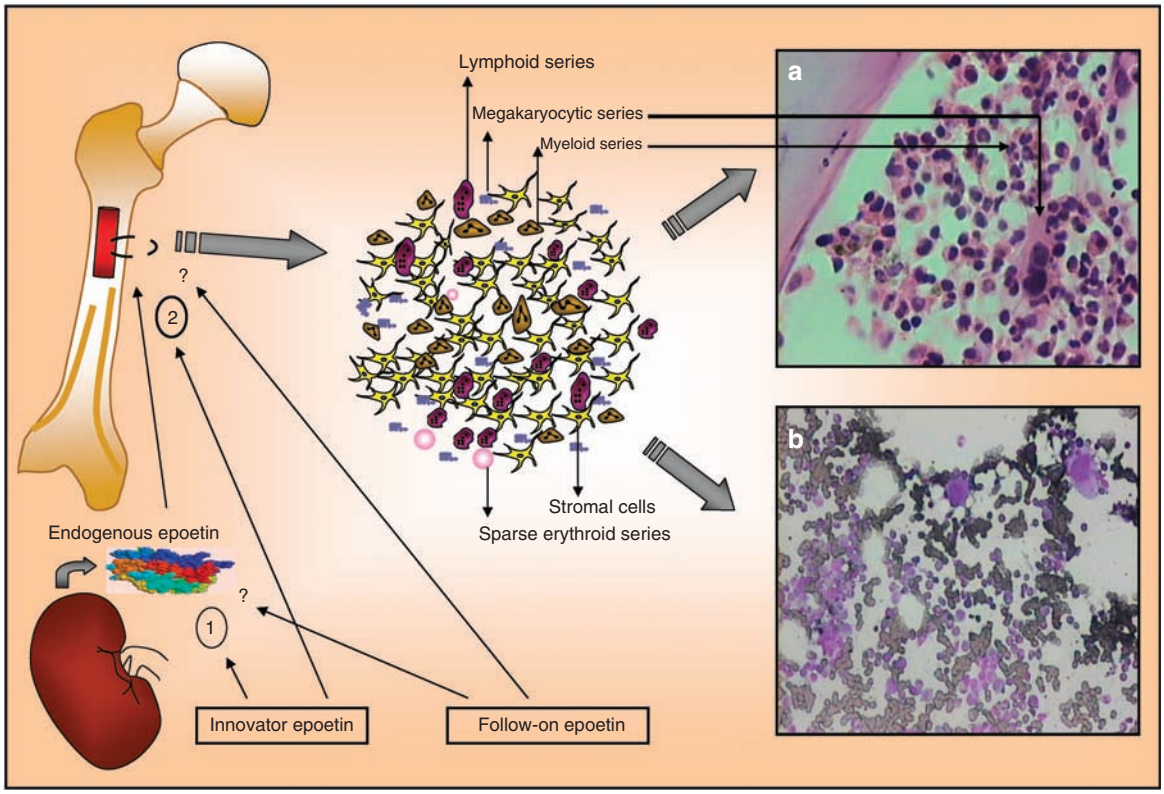

Figure 2 | A model for pathogenesis of PRCA due to innovator and follow-on epoetins with bone marrow findings of the index patient. PRCA can develop due to innovator epoetin through two mechanisms. (1) Development of cross-reacting antibodies prevents both endogenous epoetin and administered epoetin from binding to its receptor. (2) As yet, unknown factors suppressing erythroid precursors in the bone marrow. The insets are the pictures of the index patient. The bone marrow specimen obtained by trephine biopsy (panel a hematoxylin and eosin stain) is normocellular, with a marked erythroid hypoplasia, including the absence of early forms and contains megakaryocytes and myeloid elements. The bone marrow aspirate showing the same findings (panel b Giemsa stain).

Table 1 | Causes of PRCA in adults[24]

Drugs	Hematological conditions/ malignancy
Phenytoin/valproic acid	Large granular lymphocyte leukemia[a]
Trimethoprim-sulfomethoxazole	Lymphoreticular malignancy
Chloramphenicol	Myelodysplastic syndrome[a]
Isoniazid	Metastatic carcinoma
Ziduvudine	Thymoma[a]
Chlorpropamide	Systemic lupus erythematosus[a,b]
Azathioprine	Rheumatoid arthritis[a,b]
Procainamide	Juvenile rheumatoid arthritis[a,b]
Recombinant human erythropoietin	Multiple endocrine insufficiency syndromes[a,b]
Infections	
Parvovirus B19 infection	
Hepatitis (pregnancy)	
Adult T-cell leukemia virus (idiopathic[a])	
Epstein–Barr virus (idiopathic[a])	

PRCA, pure red cell aplasia.
[a]Important causes of PRCA.
[b]Autoimmune diseases.

PURE RED CELL APLASIA SECONDARY TO EPOETIN

Pure red cell aplasia (PRCA) is syndrome of marrow failure characterized by the selective reduction or absence of erythroid precursors. There is maturation arrest at the basophilic pronormoblastic phase in the bone marrow that is associated with reticulocytopenia and anemia. PRCA was originally described by Kaznelson[1] in 1922 as an entity distinct from aplastic anemia. In the 1930s, Krantz *et al.*[2], characterized the immune mechanisms for the PRCA by identifying anti-erythroid precursor cell antibodies and T-cell-mediated inhibition of erythropoiesis. Two forms of PRCA have been reported: acquired and congenital. The congenital form of PRCA, also termed Diamond–Blackfan anemia, is sporadic in onset and presents in childhood with severe anemia. It is hypothesized that these patients have poor differentiation of red cell precursors. More than 50% of these patients demonstrate craniofacial, thumb, or neck anomalies and genitourinary malformations.[3] Many of these patients respond to corticosteroids. The acquired form of PRCA may result from various etiologies (Table 1). Although most cases are considered idiopathic, in a series by Lacy *et al.*[4], T-cell large granular lymphocytic leukemia, thymoma, chronic lymphocytic leukemia, and non-Hodgkin's lymphoma were among the commonest causes in 47 adult patients with acquired PRCA. The pathogenesis of PRCA in these patients is not clear. Some of the suppressive effects on erythroid progenitors in PRCA are mediated by IgG autoantibodies or cytotoxic T lymphocytes.[4] Among the drug-related causes, PRCA is most frequently the result of an idiosyncratic reaction with phenytoin.

Innovator epoetin (Epoetin-alfa; Epogen) was introduced by Amgen (Amagen Inc., thousand oaks, CA, USA) in 1989 for the treatment of chronic kidney disease anemia. Initially, cases of PRCA were anecdotally reported.[5] Subsequently, Casadevall *et al.*[6], published the Food and Drug Administration (FDA) Med Watch data on a series of patients with epoetin-related PRCA. Most cases of PRCA have been reported from outside the United States with epoetin-alfa and have been attributed to the substitution of Tween in place of human serum albumin as a stabilizer for epoetin (of note, Tween 80 is still used as a stabilizer for non-US epoetin-alfa and Tween-20 for epoetin-beta). Although epoetin-related PRCA has been reported in patients treated with other innovator epoetin molecules, including darbepoetin alfa and epoetin-beta, the frequency was much lower than epoetin-alfa, and remained constant through the years. The interaction of Tween and the uncoated rubber in pre-filled syringes appears to cause leachates. These leachates have been implicated in causing aggregation of epoetin molecules that then enhance their antigencity.[7,8] Although these patients develop antibodies to the exogenously administered epoetin that cross-react with the endogenous epoetin, they also have factors that inhibit erythroid precursors.[6] It is hypothesized that neutralizing anti-erythropoietin antibodies block the interaction between erythropoietin and its receptor, leading to ineffectiveness of the circulating erythropoietin. Furthermore, it is postulated that the production of anti-erythropoietin antibodies against endogenous epoetin occurs more frequently in patients administered exogenous epoetin. It is postulated that anti-erythropoietin antibodies cross-react against the protein component of the exogenous recombinant epoetin. Other possible mechanisms that have been raised include interruption of the cold chain, presumably resulting in modification in the folding of the epoetin molecule, and the use of the subcutaneous route of administration. Subcutaneous administration of epoetin also appears to contribute to the development of PRCA.[6] Nevertheless, following modifications in the manufacturing process, reinforcement of cold chain, and decrease in the subcutaneous administration, there has been a dramatic reduction ($>80\%$) in PRCA.[9] It is intriguing that most epoetin-associated PRCA cases are reported in patients with chronic kidney disease and not those treated for anemia of chronic disease of other etiologies. In addition, not all patients with chronic kidney disease develop PRCA, suggesting that there may be some genetic predisposition to the susceptibility to PRCA. Alternatively, epoetin aggregation from leachates may not represent the complete explanation for the mechanism of PRCA and additional pathways for PRCA will need to be sought out.

DIAGNOSIS OF EPOETIN-RELATED PRCA

The 2006 Kidney/Dialysis Outcome Quality Initiative (K/DOQI) guidelines, which are similar to the European Best Practice Guidelines published in 2004, suggest that PRCA should be suspected in patients receiving erythropoiesis stimulating agents therapy for more than 4 weeks who develop a sudden rapid decline in Hgb level (at the rate of 0.5–1.0 g per 100 ml per week), or require red blood cell transfusions at a rate of approximately 1–2 per week in the

context of normal platelet and white blood cell counts and an absolute reticulocyte count less than 10,000/μl.[10,11]

The diagnosis of PRCA is usually made from the examination of the peripheral smear and the bone marrow aspirate. The peripheral smear shows normocytic anemia and the absence of polychromasia with a reticulocyte count of $<3\%$ or 10,000/μl. Monitoring of serial reticulocyte counts in patients suspected of PRCA is helpful in early diagnosis of PRCA. The bone marrow aspirate demonstrates the presence of maturation arrest of the erythroid series at the level of basophilic pronormoblast phase with preserved myelopoiesis and megakaryopoiesis. Bone marrow trephine has a higher yield than the bone marrow aspirate, and is also helpful in diagnosing secondary causes of PRCA, such as Parvovirus B19 infection (giant pronormoblast), lymphomas and metastatic carcinomas, and myelodysplastic syndromes. As there is inadequate utilization of iron, serum ferritin and transferrin saturation are frequently very high and are characteristic of this condition.

In patients on epoetin, the presence of anti-erythropoietin antibodies coupled with the presence of typical bone marrow findings is usually sufficient to make the diagnosis of epoetin-related PRCA. The assays used for the detection of anti-erythropoietin antibodies can be broadly divided into *in vitro* assays, such as antibody detection and functional assays, and *in vivo* assays. The following three assays: RIPA, the enzyme-linked immunosorbent assays, and the biosensor immunoassay are the most commonly used *in vitro*-neutralizing antibody detection assays. RIPA is considered the most specific and sensitive, and most commonly recommended test for detection of anti-erythropoietin antibodies. RIPA is based on competitive inhibition. The results for this assay are given for three individual determinations of anti-EPO antibody levels. A positive antibody is $\geqslant 0.9\%$ c.p.m. bound in the above assay. A negative antibody is $\leqslant 0.6\%$ c.p.m. bound in the above assay. Results that are between positive and negative are considered borderline. This test is not only specific but also very sensitive, as it can detect the presence of high-affinity anti-EPO IgG at a concentration as low as 10 ng/ml. Enzyme-linked immunosorbant assays is a quantitative method with easy implementation due to the simplicity of the procedure. However, high interassay variability and dependence on the laboratory conditions make it less sensitive than RIPA. The introduction of the 'bridging ELISA' has only modestly increased the specificity of the test. A biosensor immunoassay has recently been used for the detection of EPO antibodies. It is useful for characterization of the antibodies such as affinity and isotype. The major disadvantages are that it is expensive and its specificity (may detect non-pathogenic antibodies). The neutralizing capacity of anti-erythropoietin antibodies is best assessed by the cell proliferation assays.

FOLLOW-ON BIOLOGICS

In the developing world and more recently in Europe, follow-on biologics are available for the treatment of anemia of kidney disease. These follow-on biologics are proteins that are similar but not identical to an already licensed or approved innovator biologic (the innovator biologic) with respect to bioequivalence and/or therapeutic equivalence. Innovator biologics are developed through biotechnology practices, including recombinant human technology, gene technology, or antibody methods. Each area of the manufacturing process of a biopharmaceutical, such as choice of the expression vector, cell fermentation, host cell line, protein purification protocols, sterile filling, and drug product formulation, is a determining factor.[12] Subtle variations in temperature, cell culture conditions, or even transport or storage conditions may result in significant changes in the clinical efficacy, biological activity, or immunogenicity of the product in some cases.[13–15] Thus, follow-on biologics, such as FOEs, originating from different manufacturers may be associated with subtle differences in their biochemical composition, which could translate into therapeutic differences.[16,17] In addition, the differences in conformation of the biologic could potentially impact on their immunogenicity.

FOLLOW-ON EPOETINS

FOEs are targeted to mimic the action of the original innovator epoetins. FOEs are frequently produced by local biotechnology companies and licensed by the local country's FDA or equivalent drug regulatory agency. In developing countries, FOEs are an inexpensive substitute for innovator epoetins. Indeed, the price may be discounted as much as 60% over the cost of the innovator epoetin. On the other hand, in Europe, FOEs are likely to be priced more closely to the innovator epoetin. Hitherto, prior to the patient described here, PRCA had not been reported with FOEs. However, FOEs may differ from the innovator biologic with respect to tertiary or quaternary folding, the cell lines used to express the protein, glycosylation content, and the degree of aggregation and isomer content. Others have also documented variable physical characteristics, such as pH, osmolality, and isoelectric charge, with FOE[18,19]. These subtle differences could provide plausible explanations for potential immunogenicity for FOEs. Indeed, it is most likely that the same mechanisms that accounted for the immunogenicity of innovator epoetin could also explain the immunogenicity of FOEs.

Each manufacturer of a FOE develops their own manufacturing processes and has their own quality surveillance protocols; this has the potential to generate subtle differences that could impact on the content and activity of the biologic. With the inability to reproduce identical molecule, efficacy measures and standardized analytical and pre-clinical tests for FOEs have been insufficient to demonstrate the comparability of two biological products.[19,20] In a study that we have conducted, FOE samples procured from pharmacies in Korea, Thailand, Vietnam, India, Philippines, Indonesia, Iran, Yemen, Jordan, Lebanon, and Columbia revealed variable physical characteristics with respect to pH, osmolality, and isoelectric charge. Some of

Table 2 | Algorithm for PRCA due to innovator/follow-on epoetin

I. Suspect a case
Patient on epoetin for $\geqslant$4 weeks
Severe or progressively worsening anemia
Reticulocytopenia
Normal platelet and leukocyte counts
Requirement for frequent blood transfusions
II. Diagnosis
Bone marrow biopsy
Anti-erythropoietin antibodies by RIPA
Increased transferrin saturation or ferritin
III. Treatment
Withdrawal of erythropoietin
Renal transplantation
Prednisone $\pm$ cyclophosphamide

PRCA, pure red cell aplasia; RIPA, radioimmunoprecipitation assay.

these FOEs revealed varying potencies, higher endotoxins, and aggregates compared with epoetin-alfa.[18] Since the European Union (EU) has developed more stringent standards for the monitoring of quality, it is unlikely that this variability in quality and problems with endotoxin contamination will be observed with FOEs that are marketed in Europe, although pharmacovigilance is still warranted. An inherent risk involved in the use of any biologic is its immunogenic potential. Immunogenicity can be due to many factors, such as altered chemical structure of the molecule, physical degradation, and chemical decomposition, apart from an effect of protein purification and product formulation.[21] The role of epoetin aggregates, leachates from rubber lined pre-filled syringes, and the subcutaneous route of administration have all been implicated in the etiology of PRCA secondary to innovator epoetins. Although FOEs are often used by practitioners for economic reasons, the varying potency observed in tested lots of the FOE raises questions about whether there is sufficient scrutiny of FOEs that are currently being marketed, especially in developing nations.

TREATMENT OF EPOETIN-RELATED PRCA

There is no consensus on optimal therapy for PRCA. As in our patient, withdrawal of all kinds of erythropoiesis-stimulating agents was the critical step because prior observations demonstrate that the cross-reacting antibodies bind the diverse range of epoetin molecules, including darbepoetin alfa.[22] Despite reports of allergic reactions after re-introduction of erythropoietin, studies suggested success with reintroduction of epoetin after complete disappearance of anti-erythropoietin antibodies.[23] The ESRD patient in whom kidney transplantation is not feasible is especially challenging from a management standpoint. Although spontaneous remissions are rare, immunosuppressive treatment accelerates recovery from erythropoietin-induced PRCA.[23,24] In these patients, there is an agreement that long-term treatment with steroids and cyclophosphamide is warranted. Other treatment modalities include plasmapheresis, cyclosporine, intravenous gamma globulins, and rituximab. Hematide is a pegylated synthetic peptide, which has been developed as an erythropoiesis-stimulating agent. No reports of PRCA have appeared in the literature, perhaps because hematide has novel amino acid sequences that are not related to endogenous erythropoietin. Although preliminary studies in rat models with hematide were promising with the correction of antibody-induced anemia (rat PRCA model), studies with long-term follow-up, especially in patients with chronic kidney disease, are not available.[25,26] An algorithm based on the recommendation of the 2004 Revised European Best Practice Guidelines for the Management of Anaemia in Patients with Chronic Renal Failure is described in Table 2 (specific hematologic and/or bone marrow criteria are detailed in the original guideline document).[10,11]

In summary, with the patent expiry of innovator epoetins, FOEs will be an important option for the treatment of chronic kidney disease anemia. Physicians must be vigilant because FOEs may result in PRCA. Furthermore, although pharmacovigilance is a daunting and potentially expensive task for drug regulatory authorities, especially for countries in the developing world, scrutiny of the manufacturing and storage processes for epoetin is essential. Perhaps even more important in ensuring the long-term safety of biologic products, especially FOEs, is the development of a post-marketing surveillance infrastructure that allows for pharmacovigilance. It seems a price worth paying.

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