



EXCEPTIONAL CASE

Pure red cell aplasia induced by epoetin zeta

Vincenzo Panichi¹, Guido Ricchiuti¹, Alessia Scatena¹, Lucia Del Vecchio² and Francesco Locatelli²

¹Department of Nephrology and Dialysis, Versilia Hospital, Lido di Camaiore (LU), Italy and ²Department of Nephrology and Dialysis, A. Manzoni Hospital, Lecco, Italy

Correspondence and offprint requests to: Lucia Del Vecchio; E-mail: l.delvecchio@ospedale.lecco.it

Abstract

Pure red cell aplasia (PRCA) may develop in patients with chronic kidney disease receiving erythropoiesis-stimulating agents (ESA). We report on a 72-year-old patient who developed hypo-proliferative anaemia unresponsive to ESA following the administration of epoetin zeta subcutaneously for 7 months. On the basis of severe isolated hypoplasia of the erythroid line in the bone marrow and high-titre neutralizing anti-erythropoietin antibodies (Ab), a diagnosis of Ab-mediated PRCA was made. Epoetin zeta was discontinued and the patient was given steroids. This was associated with anaemia recovery. To our knowledge this is the first PRCA case related to epoetin zeta.

Key words: anaemia, chronic kidney disease, epoetin zeta, erythropoietin, pure red cell aplasia

Introduction

Pure red cell aplasia (PRCA) is a rare adverse reaction occurring in patients with chronic kidney disease (CKD) who are treated with erythropoiesis-stimulating agents (ESA). It is generated by epoetin-induced antibodies (Ab) that neutralize all the exogenous erythropoietin (EPO) and cross-react with endogenous EPO.

In the early 2000s a peak increase in the incidence rate of PRCA was observed after a change in the formulation of epoetin alpha produced outside the USA [1]. Following reinforcement of the cold storage chain, a shift towards the intravenous administration route and the elimination of uncoated rubber stoppers, the incidence of PRCA has markedly decreased [2].

Biosimilars of epoetin alpha have been approved in the European Union since 2007, and following the expiration of the US patent, they will enter the US market soon. Concerns about immunogenicity have been raised also for these drugs [3].

We report on a patient who developed PRCA following the subcutaneous administration of epoetin zeta, which is one of the two biosimilars of epoetin alpha licensed in Europe.

To our knowledge this is the first PRCA case related to epoetin zeta.

Case report

A 72-year-old Caucasian female was regularly followed up in the Nephrology Outpatient Clinic of Versilia Hospital [Lido di Camaiore (LU), Italy] because of a slowly progressive CKD.

In 1990 the patient underwent left nephrectomy for severe nephrolithiasis, complicated by acute pyelonephritis and sepsis. In 1991, she developed new stones in the right kidney and received extracorporeal shock wave lithotripsy. In 1995 right nephrostomy was put in place because of obstructive nephrolithiasis. In 2006 hypertension was detected. In 2007 she had acute myocardial infarction. Since then, she has been under regular cardiologic follow-up. From 2007 until 2014 kidney function slowly deteriorated, reaching stage IV–V CKD.

In June 2014, she attended the Day Hospital of the Nephrology Unit for severe hip arthrosis. Her (Hb) was 9.7 g/dL with adequate

Received: February 16, 2016. Accepted: April 4, 2016

© The Author 2016. Published by Oxford University Press on behalf of ERA-EDTA.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

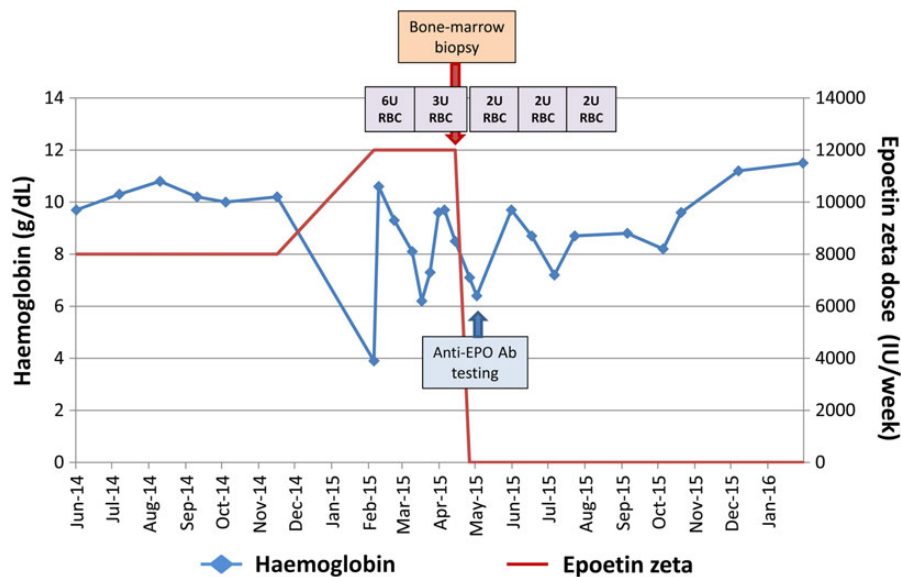


Fig. 1: Trend of haemoglobin levels and epoetin doses over time. RBC, red blood cells.

Table 1. Main laboratory parameters at onset of PRCA

	25/02/2015	09/04/2015
Haemoglobin (g/dL)	3.9–9.3 ^a	6.2–7.3 ^a
Reticulocytes (/μL)	NA	2500
Leucocytes (N/mm ³)	6850	8680
Platelet count	136 000	137 000
Serum iron (μg/dL)	227	218
Transferrin saturation (%)	80.5 ^a	82.2 ^a
Serum ferritin (ng/mL)	268	512
Serum creatinine (mg/dL)	3.5	3.7
C-reactive protein (mg/L)	NA	0.3
Parathyroid hormone (pg/mL)	NA	155

NA, not available.

^aPost-transfusion.

iron stores; she was prescribed epoetin zeta 4000 IU twice a week subcutaneously (Figure 1) and analgic therapy for hip pain.

In December 2014, she was hospitalized for 4 days in the Nephrology Department due to the persistence of severe hip pain. The patient's serum creatinine was 3.7 mg/dL and Hb was 10.2 g/dL. Given that occult blood was detected in three stool samples, colonoscopy was suggested.

In February 2015, the patient arrived at the Emergency Unit because of severe asthenia and mild shortness of breath. Her Hb was 3.9 g/dL. She received blood transfusion (six units of packed red cells) and was admitted again to the Nephrology Department. Unremarkable findings were obtained from chest computed tomography, bronchoscopy and upper endoscopy. Colonoscopy showed diverticulosis, but was not diagnostic for incomplete cleaning. At 2 weeks after admission, at discharge her Hb was 9.3 g/dL; epoetin zeta dose was increased to 4000 IU three times per week.

In April 2015, she was hospitalized again for 8 days because of severe anaemia (Hb 6.2 g/dL, reticulocytes 0.1%, 2500/μL). The other main laboratory findings are summarized in Table 1. Colonoscopy was repeated confirming known diverticulosis. Abdomen ultrasound was unremarkable for possible causes of anaemia.

The patient underwent bone marrow biopsy showing severe hypoplasia of the erythroid line (CD34 blasts <5%) with hyperplasia of the megakaryocytic and granulocytic lines.

In May 2015, at the time when bone marrow biopsy became available, the patient had become transfusion dependent and epoetin zeta administrations were immediately interrupted. Assays for antinuclear Ab, anti-DNA Ab and hepatitis B Ab were normal; Parvovirus B19 DNA and hepatitis B antigen were not detected. Anti-EPO Ab were tested by IPM Biotech GmbH, Hamburg, Germany and found positive in three different samples by a screening assay, followed by specificity confirmation testing. These were anti-EPO neutralizing Ab (titre of 1:81 920). She was then diagnosed with anti-EPO-mediated PRCA.

The patient was prescribed oral prednisone (0.5 mg/kg/day) for 3 months and progressive decrease in the need for blood transfusions was observed. In September 2015, she received her last blood transfusion and steroid therapy was interrupted. Since then, her Hb values have stabilized (last available Hb value of 11.5 g/dL in February 2016). No worsening of renal function had occurred over the period (in February 2016, serum creatinine was 3.9 mg/dL). Retesting of anti-EPO Ab titre has already been planned, in the case the patient needs re-challenging with ESA in the future.

Discussion

In the late 1980s, epoetin alfa was developed thanks to the recombinant DNA technique in Chinese hamster ovary cells. This is a delicate manufacturing process, which invariably leads to heterogeneity of the final product. Given that the drug is poorly water-soluble, excipients are required to enhance dispersion or inhibit precipitation when mixed with water. As a result, all ESA molecules are delicate and fragile, require cold storage and are sensitive to changes in the stabilizer and/or manufacturing processes. Of note, the interruption of the cold chain may be less problematic with long-acting ESA, as they have a longer stability at room temperature compared with short-acting ESA. Experience has shown that even minor modifications can increase immunogenicity and cause PRCA. This is particularly

true for the subcutaneous administration route. The upsurge of PRCA cases with Eprex[®] was preceded by the substitution of human serum albumin with polysorbate 80 [4]. The hypothesis that this stabilizer may elicit the formation of immunogenic epoetin-containing micelles, possibly increasing immunogenicity [5], has been questioned [6]. The uncoated rubber stoppers in the pre-filled syringes could have possibly increased immunogenicity as well. The number of reported cases of PRCA has sharply decreased since then [2]. This may be due to the shift from the subcutaneous to the intravenous route of administration, the reinforcement of the product cold chain or the elimination of uncoated rubber syringe stoppers. In 2008 regulatory authorities readmitted the subcutaneous use of Eprex[®] in the absence of a vascular access for intravenous administration. More recently, an unexpected increase in local cases of PRCA associated with subcutaneous administration of Eprex[®] was described in Singapore [7].

The introduction of biosimilars in the EU market has brought savings of around 15–30%, together with a price reduction of the originator. However, concerns about immunogenicity have not been dissipated yet. Considering that the manufacturing process of epoetin alfa is owned by the producer of the reference product and cannot be precisely duplicated, companies developing biosimilars have to implement a new manufacturing process. This is true also in highly regulated countries such as those of the EU, or the USA, where high-quality processes are used.

Some years ago, one randomized clinical trial using HX575 was halted for safety reasons following one PRCA case and one anti-EPO Ab positivity [3]. The potential cause of immunogenicity was identified in soluble tungsten, most likely derived from the pins used to manufacture the syringes, causing unfolding and aggregation of HX575 in pre-filled syringes [8]. Considering that only 160 patients were treated with HX575 in the trial over a follow-up of 52 weeks, the incidence of anti-EPO neutralizing Ab in this study is particularly high.

Epoetin theta is an analogue of epoetin beta, which was approved as an originator by European Medicine Agency in 2009. A PRCA case has also recently been reported with this molecule [9].

Data from one clinical trial administering epoetin zeta subcutaneously to 230 CKD patients did not report cases of anti-epoetin Ab or PRCA [10]. Similarly, no cases had occurred during a large post-authorization observational study involving more than 1600 CKD patients receiving epoetin zeta intravenously [11]. Our patient had been enrolled in a second, ongoing, post-authorization observational study to estimate the incidence of PRCA and/or neutralizing Ab during treatment with epoetin zeta administered subcutaneously in more than 6000 CKD patients [Post-Authorisation Safety Cohort Observation of Retacrit[™] (Epoetin Zeta) Administered Subcutaneously for the Treatment of Renal Anaemia, PASCO II].

To our knowledge, this is the first PRCA case that has been described for epoetin zeta.

Our patient suddenly developed severe hypo-proliferative anaemia after 7 months from the start of epoetin zeta administered subcutaneously. This is in line with data in the literature describing a median of 7 months (range 1 month to 5 years) from the beginning of therapy until the diagnosis of PRCA [1].

The severity of anaemia, which had become unresponsive to ESA therapy, is testified by the very low reticulocyte count and by the dependence on blood transfusions. The platelet and granulocyte counts were normal. PRCA diagnosis was supported by the findings of the bone marrow biopsy, showing severe hypoplasia of the erythroid line, and by the presence of high-titre anti-EPO neutralizing Ab. No other causes of PRCA, such as Parvovirus or

hepatitis B infection, thymoma, lymphoproliferative disorders, drugs or autoimmune disease were identified. PRCA has also been reported in patients with no underlying disease [12]. However, the timing from drug exposure and the fact that the patient received uniquely epoetin zeta makes the relationship between the drug and the occurrence of PRCA extremely likely.

PRCA has been described to occur with all ESA [2]. Considering its rarity, it is difficult to distinguish whether this is a sporadic case or the expression of increased immunogenicity of epoetin zeta. The calculation of PRCA incidence based on length of exposure to epoetin zeta would be of interest, but is beyond the scope of this case report.

Various immunosuppressive strategies have been used to cure PRCA [13, 14]. However, in the absence of randomized clinical trial, there are insufficient data to provide guidance on the preferred immunosuppressive agents or treatment regimen [14]. Our patient obtained remission from PRCA following therapy with steroids. This is in line with other reports in the literature [14, 15]. This conservative strategy was chosen considering her age and comorbidities. Interestingly, after therapy her Hb stabilized without the need to challenge her with new ESA therapy. It is possible that the iron load she received with blood transfusion had contributed to Hb stability after the recovery of PRCA.

One possible limitation of this case report is that reticulocytes were not tested at onset and that PRCA diagnosis was made with a certain delay. While this does not reduce the importance of the relationship between epoetin zeta use and the occurrence of PRCA, we acknowledge that an earlier testing of reticulocyte values would have prevented needless workup of anaemia with endoscopies and CT scans, and directed us towards bone marrow biopsy sooner. A more timely diagnosis would also have prevented the increase in epoetin zeta dose, which have possibly enhanced anti-EPO Ab production.

To conclude, we report for the first time a patient who developed Ab-mediated PRCA after receiving epoetin zeta subcutaneously.

Conflict of interest statement

The patient described in this paper was enrolled in the post-marketing observational study EPOE-09-11 PASCO II, which is sponsored by Hospira. Hospira has been informed about this case and the intention to publish it. V.P., G.R. and A.S. have no conflict of interest to declare. L.D.V. was a member of an Advisory Board supported by Astellas. F.L. was a member of an Advisory Board and/or speaker at meetings supported by Akebia, Amgen, Astellas, Janssen, GSK, Hospira, Pharmacosmos, Roche and Sandoz.

References

- Gershon SK, Luksenburg H, Cote TR et al. Pure red-cell aplasia and recombinant erythropoietin. *N Engl J Med* 2002; 346: 1584–1586
- Macdougall IC, Casadevall N, Locatelli F et al. PRIMs study group. Incidence of erythropoietin antibody-mediated pure red cell aplasia: the Prospective Immunogenicity Surveillance Registry (PRIMS). *Nephrol Dial Transplant* 2015; 30: 451–460
- Haag-Weber M, Eckardt KU, Hörl WH et al. Safety, immunogenicity and efficacy of subcutaneous biosimilar epoetin- α (HX575) in non-dialysis patients with renal anemia: a multi-center, randomized, double-blind study. *Clin Nephrol* 2012; 77: 8–17

4. Casadevall N, Nataf J, Viron B et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. *N Engl J Med* 2002; 346: 469–475
5. Hermeling S, Schellekens H, Crommelin DJ et al. Micelle-associated protein in epoetin formulations: a risk factor for immunogenicity? *Pharm Res* 2003; 20: 1903–1907
6. Macdougall IC, Roger SD, de Francisco A et al. Antibody-mediated pure red cell aplasia in chronic kidney disease patients receiving erythropoiesis-stimulating agents: new insights. *Kidney Int* 2012; 81: 727–732
7. Health Sciences Authority. http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Safety_Information_and_Product_Recalls/Product_Safety_Alerts/2013/increase_in_antibody-mediated.html. (15 January 2016, date last accessed)
8. Seidl A, Hainzl O, Richter M et al. Tungsten-induced denaturation and aggregation of epoetin alfa during primary packaging as a cause of immunogenicity. *Pharm Res* 2012; 29: 1454–1146
9. Herrington W, Wieser C, Rosenkranz AR. Pure red cell aplasia after treatment of renal anaemia with epoetin theta. *Clin Kidney J* 2013; 6: 539–542
10. Krivoshiev S, Wizemann V, Czekalski S et al. Epoetin Zeta Study Group. Therapeutic equivalence of epoetin zeta and alfa, administered subcutaneously, for maintenance treatment of renal anemia. *Adv Ther* 2010; 27: 105–117
11. Dellanna F, Fluck RJ, Lonnemann G et al. Results from a safety cohort of patients with renal anemia receiving the biosimilar epoetin zeta: The PASCO I study. *Clin Nephrol* 2015; 84: 280–288
12. Kwong YL, Wong KF, Liang RHS et al. Pure red cell aplasia: clinical features and treatment results in 16 cases. *Ann Hematol* 1996; 72: 137–140
13. Verhelst D, Rossert J, Casadevall N et al. Treatment of erythropoietin-induced pure red cell aplasia: a retrospective study. *Lancet* 2004; 363: 1768–1771
14. Bennett CL, Courmoyer D, Carson KR et al. Long-term outcome of individuals with pure red cell aplasia and antierythropoietin antibodies in patients treated with recombinant epoetin: a follow-up report from the Research on Adverse Drug Events and Reports (RADAR) Project. *Blood* 2005; 106: 3343–3347
15. Zerbi S, Cozzi G, Mercieri A et al. Successful corticosteroid therapy for epoetin-beta-induced pure red-cell aplasia in a hemodialysis patient. *Am J Hematol* 2006; 81: 803–804