

Teaching Point (Section Editor: W. Herrington)

Pure red cell aplasia after treatment of renal anaemia with epoetin theta

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Introduction

The treatment of renal anaemia in patients with chronic kidney disease (CKD) has always been a challenge. The introduction of erythropoiesis-stimulating agents (ESAs) appeared to be a milestone, enabling physicians to avoid red blood cell transfusions in this patient group. In the last decade, however, these assumed advances were tarnished by the occurrence of a new condition: ESA-induced, antibody-mediated, pure red cell aplasia (PRCA). PRCA is characterized by the combination of anaemia, low reticulocyte count, absence of erythroblasts in the bone marrow, resistance to therapy with ESAs and detection of neutralizing antibodies against erythropoietin.

The exposure-adjusted incidence of 0.02–0.03 per 10 000 patient years is considered rare. In the years 2002 and 2003, it peaked, however, at an incidence of 4.5 cases per 10 000 patient years, mainly caused by a preparation of epoetin alfa [1]. Hypothetic explanations concerning the PRCA-inducing potential of this specific preparation of epoetin alfa have been published [1]. Nevertheless, we have to accept the possibility that all ESAs can induce an immunological response in the form of neutralizing antibodies [2, 3]. Because of the complexities of manufacturing biopharmaceuticals, there are safety concerns regarding me-too biologicals and biosimilars and the automatic substitution of originator drugs for economic reasons [4, 5]. In the interest of patient safety, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines only recommend the use of biosimilars approved by an independent regulatory agency that are subject to pharmacovigilance plans [6].

We report on a patient treated with the originator drugs such as epoetin theta, epoetin beta and darbepoetin alfa, who developed ESA-induced PRCA. The repetitive switching of agents hampered our ability to attribute PRCA to the appropriate agent.

Case description

A male patient, born in 1943, first presented in our nephrology unit in 2006 with glomerulonephritis [nephritic

urine, hypertension, creatinine 1.90 mg/dL (168 µmol/L), haemoglobin (Hb) 14.3 g/dL (143 g/L), Figure 1], refusing a morphological diagnosis (kidney biopsy). The patient returned in 2009, again presenting with nephritic urine, creatinine at 4.56 mg/dL (403 µmol/L) and Hb 12.2 g/dL (122 g/L). In 2010, there was a slow progression of renal insufficiency, with creatinine at 5.20 mg/dL (460 µmol/L) and Hb stable at >12.0 g/dL (120 g/L). In 2011, creatinine increased to 8.30 mg/dL (734 µmol/L), with decreasing Hb levels. In May 2011, Hb was at 10.3 g/dL (103 g/L). A normochromic, normocytic blood count was unsuspecting of iron deficiency. We started subcutaneous (s.c.) erythropoietin substitution using epoetin theta. Hb rapidly increased to 12.0 g/dL (120 g/L), where we stopped epoetin theta administration upon reaching the upper limit of the Hb target. In October 2011, Hb had again decreased severely to Hb 9.3 g/dL (93 g/L), triggering a new sequence of epoetin theta. Because of the beginning of uraemic symptoms, we started renal replacement therapy, choosing continuous ambulatory peritoneal dialysis (CAPD). After initiation of CAPD, we observed a continuous decrease of Hb to 7.9 g/dL (79 g/L), despite an increase of epoetin theta dose. A detailed workup was initiated to identify reasons for the Hb non-response, showing an iron status of total iron 66 µg/dL (11.81 µmol/L), transferrin level 168 µg/dL (19.09 µmol/L), transferrin saturation 27.9% and ferritin 358 µg/mL (755.38 pmol/L). Reticulocyte counts remained constantly low with levels of ≤1.2% (proportion of red blood cells of ≤0.012). A bone marrow sample showed a hyporegenerative state. We tested for anti-erythropoietin antibodies using radioimmunoprecipitation (RIP), with negative results (Laborinstitut Prof. Seelig, Karlsruhe, Germany).

Subsequently, the patient changed to a different dialysis centre, where he was switched to epoetin beta. Since the patient self-administered the product, he was further switched to the longer-acting, subcutaneously administrable darbepoetin alfa. Both epoetin beta and darbepoetin alfa were unable to improve the patient's Hb. In May 2012, we repeated the test for anti-erythropoietin antibodies in two different laboratories: (i) Laborinstitut Prof. Seelig, Karlsruhe, Germany (RIP), and (ii) IPM Biotech,

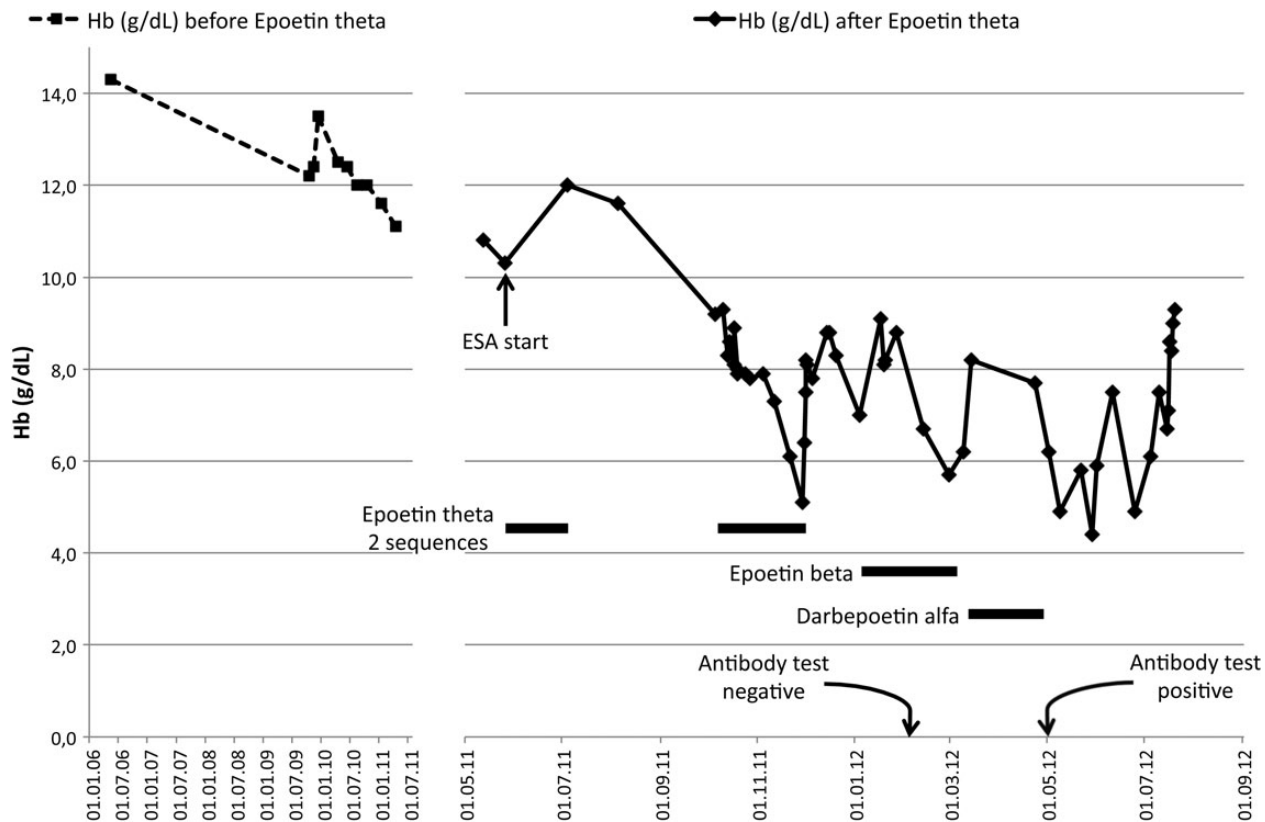


Fig. 1. Hb trajectory and treatment history. ESA, erythropoiesis-stimulating agent.

Hamburg, Germany (bioassay using the human erythro-leukaemia cell line TF-1; see Supplementary Material for a description of the testing procedure). Both antibody tests were positive, with the bioassay revealing a highly positive antibody titre of 1:1600. We immediately stopped the administration of any ESA and switched the patient to red blood cell transfusions. We started immunosuppression with prednisolone 75 mg and cyclosporine A 200 mg (target cyclosporine A level: 100–150 ng/mL; 83.2–124.8 nmol/L), which resulted in a life-threatening peritonitis caused by *Prevotella melaninogenica* and *Bacteroides* sp. We stopped immunosuppressive treatment and evaluated the patient for renal transplantation after earlier refusal.

Discussion

PRCA is triggered by the development of neutralizing antibodies against endogenous erythropoietin that may cross-react with different ESAs [7]. It is assumed that all biopharmaceuticals, including protein-based ESAs, are immunogenic [5]. However, the mechanism of how the natural B-cell tolerance is broken remains to be elucidated. Two major causes have been identified: (i) changes to the three-dimensional (3D) structure of the protein (protein aggregation) and (ii) route of administration.

(i) Protein aggregates can activate autoreactive B-cells by resembling the repeated self-epitope structure of viral capsids. Protein aggregation is not fully understood, but there are three potential mechanisms. First, aggregation may be triggered by the presence of small amounts of a contaminant, such as a damaged

form of the protein itself, host cell proteins or even non-protein materials (organic leachate of uncoated rubber stoppers of prefilled syringes, polysorbate 80, tungsten). A second mechanism is partial unfolding of the native protein during storage. Some partially or fully unfolded protein molecules are always present in protein solutions, but mostly refold to their native structure. Alternatively, these unfolded proteins may co-aggregate with other such molecules or may be incorporated into an existing aggregate to form larger aggregates. Elevated temperature, shaking (shear and air-liquid interface stress), surface adsorption and other physical or chemical stress factors facilitate the aggregation of unfolded proteins. Since the patient self-administered the ESAs, it is thinkable that an interrupted cold chain had triggered this process. The interruption of the cold chain may be less problematic with long-acting ESAs, as they have a longer stability at room temperature compared with short-acting ESAs [8–11]. A third aggregation mechanism is self-association of the native protein to form oligomers. Oligomers vary with solvent conditions such as pH and ionic strength, are very difficult to detect and can become irreversible [12]. These product-related factors are of high interest, especially in consideration of the increasing number of ESAs and manufacturers entering the markets and the high economic pressure to produce and prescribe cheaper medical products. The specific demands on storage and handling of a sensitive product, such as erythropoietin, may also be too complex for—mostly elderly—patients.

(ii) The route of administration of ESAs was also related to PRCA. The s.c. route seems to evoke a stronger immune response than the intravenous (i.v.) route,

possibly due to the high concentration of antigen-presenting cells in the skin and the longer local presence of the antigen. Epidemiological data support this theory. The incidence of PRCA is estimated at 0.67 per 100 000 patient years for i.v. and 20.66 per 100 000 patient years for s.c. administration [13]. However, the s.c. route of administration has organizational benefits, especially for both CAPD and CKD patients not on dialysis.

The detection of the neutralizing antibodies was a challenge in our case. In retrospect, the Hb trajectory, the reticulocyte count and the bone marrow sample clearly identify epoetin theta as the trigger of PRCA. Nevertheless, the first examination of the patient's serum using a RIP assay was negative. Only the control tests months later (RIP and TF-1 bioassay) were positive. By this time, however, two more ESAs (epoetin beta and darbepoetin alfa) had been administered. Therefore, the sensitivity and degree of reliance of the antibody tests are essential [14]. There are several types of binding antibody assays, including enzyme-linked immunosorbent assays (ELISA), RIP, the BIAcore biosensor assay and various other bioassays, as reviewed in detail by Thorpe and Swanson [15]. ELISA assays generally have a poor ability to detect lower-affinity anti-erythropoietin antibodies with limited utility as a screening assay. RIP assays are more apt for screening purposes. They measure binding antibodies and their production in amplitude or time to appearance and have been shown to correlate with the severity of symptoms of PRCA. In our case, however, the RIP assay did not detect antibodies, despite the apparent Hb drop and non-response to epoetin theta. Bioassays are used for measuring the neutralizing capability of anti-erythropoietin antibodies. They are also used for studying the binding stoichiometries of antibodies with their target proteins [15]. In our case, a bioassay using cultures of the human erythroleukaemia cell line TF-1 was used to confirm the results of the second RIP test.

An immunological response usually develops gradually over several months and can be present before the occurrence of clinical symptoms. Additionally, the life-cycle of erythrocytes is ~2 months, making an abrupt drop in Hb a rare event, thus dampening the chances to perform clarifying tests in time. For these reasons, it is assumed that full-blown antibody-mediated PRCA may be identified as late as 1 year after the first occurrence of an immunological response. As we have observed a continuous decline in Hb early in the course of ESA treatment and started a detailed workup to detect the underlying causes at the first signs of hyporesponsiveness to epoetin theta, it is unclear, why the RIP test did not detect the presence of antibodies in our patient. It has been observed that the RIP assay is used in various laboratory conditions and formats, leading to large differences in sensitivities and specificities of different assays [15]. In our case, however, the results of the second RIP test were confirmed by the bioassay, excluding quality issues as an explanation.

Due to the wide range of marketed ESAs, including me-too biologicals and biosimilars, there is a high economic pressure on ESA manufacturers and health care providers. The reimbursement authorities expect physicians to prescribe the cheapest products and to accept repetitive switching of agents. As the presented case impressively demonstrates, it seems—from a safety point of view—preferable to avoid repetitive switching. After a series of different products has been administered, the PRCA-triggering

product can only be identified if the accompanying severe anaemia and/or antibodies have been detected before the switch. Therefore, traceability of all ESAs given to a patient is essential. Ideally, a serum sample should be stored before any switch is made, but this seems to be very difficult in clinical practice [4, 16, 17].

Conclusion

The pathogenesis of ESA-induced PRCA remains unclear. Several, partly hypothetical, causes are discussed in the literature, such as patient- or product-related factors. Additionally, treatment duration, route of administration, inappropriate transport and storage, or the frequent change of ESAs may play a role. Until the true causes of PRCA have been identified, it is necessary to be alert to the dangers of frequent, cost-driven changes in ESAs, to reconsider the route of administration and to increase our efforts in pharmacovigilance.

Teaching points

- (i) The main challenges with biopharmaceuticals—originators, me-toos and biosimilars—are variable potency and immunogenicity. These are hypothesized to be due to glycosylation, contamination and changes to 3D structure, which may occur between products and even between batches.
- (ii) PRCA is a potentially life-threatening immune response caused by neutralizing antibodies against endogenous erythropoietin that may cross-react with different ESAs. It can theoretically be induced by any protein-based ESA.
- (iii) Any product substitution should only be made with the specific approval of the prescribing physician. Procurement practices in the hospital sector should always provide for a sufficiently broad choice of products.
- (iv) In the case of PRCA, product changes may prohibit the identification of the antibody-causing product, a practice, which has to be reconsidered in the interest of pharmacovigilance.

Supplementary data

Supplementary data are available online at <http://ckj.oxfordjournals.org>.

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References

1. McKoy JM, Stonecash RE, Cournoyer D *et al.* Epoetin-associated pure red cell aplasia: past, present, and future considerations. *Transfusion* 2008; 48: 1754–1762
2. 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
3. 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

4. Covic A, Cannata-Andia J, Cancarini G *et al.* Biosimilars and biopharmaceuticals: what the nephrologists need to know—a position paper by the ERA-EDTA Council. *Nephrol Dial Transplant* 2008; 23: 3731–3737
5. Dorner T, Strand V, Castaneda-Hernandez G *et al.* The role of biosimilars in the treatment of rheumatic diseases. *Ann Rheumat Dis* 2013; 72: 322–328
6. KDIGO. KDIGO clinical practice guideline for anemia in chronic kidney disease. *Kidney Int* 2012; (Suppl 2): 279–335
7. Elliott S, Pham E, Macdougall IC. Erythropoietins: a common mechanism of action. *Exp Hematol*. 2008; 36: 1573–1584
8. Aranesp Summary of Product Characteristics. 2012. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000332/WC500026149.pdf (22 May 2013, date last accessed)
9. Mircera Summary of Product Characteristics. 2012. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000739/WC500033672.pdf (22 May 2013, date last accessed)
10. Eprex Summary of Product Characteristics. 2012. <http://www.medicines.org.uk/emc/medicine/889/SPC/Eprex+2000%2c+4000+and+10000+IU+ml+solution+for+injection+in+pre-filled+syringe> (22 May 2013, date last accessed)
11. NeoRecormon Summary of Product Characteristics. 2012. <http://www.medicines.org.uk/emc/medicine/7747/SPC/Neorecormon+Solution+for+Injection+in+Pre-Filled+Syringe> (22 May 2013, date last accessed)
12. Arakawa T, Philo JS, Ejima D *et al.* Aggregation analysis of therapeutic proteins: part 1. *Bio Process Int* 2006; 4: 32–42
13. Locatelli F, Aljama P, Barany P *et al.* Erythropoiesis-stimulating agents and antibody-mediated pure red-cell aplasia: here are we now and where do we go from here? *Nephrol Dial Transplant* 2004; 19: 288–293
14. Kromminga A, Schellekens H. Antibodies against erythropoietin and other protein-based therapeutics: an overview. *Ann NY Acad Sci* 2005; 1050: 257–265
15. Thorpe R, Swanson SJ. Current methods for detecting antibodies against erythropoietin and other recombinant proteins. *Clin Diagn Lab Immunol*. 2005; 12: 28–39
16. Casadevall N. Immune-Response and Adverse Reactions: PRCA Case Example. 2009. http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2009/11/WC500011064.pdf (22 May 2013, date last accessed)
17. Cournoyer D, Toffelmire EB, Wells GA *et al.* Anti-erythropoietin antibody-mediated pure red cell aplasia after treatment with recombinant erythropoietin products: recommendations for minimization of risk. *J Am Soc Nephrol* 2004; 15: 2728–2734

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