Case Report

Antibody-mediated pure red cell aplasia in a dialysis patient receiving darbepoetin alfa as the sole erythropoietic agent

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Introduction

The introduction of recombinant human erythropoietin (epoetin) transformed the management of anaemia for the vast majority of dialysis patients. The immediate benefit of transfusion-independence was a lower risk of contracting blood-borne infections and iron overload. The raised haematocrit led to improvements in quality of life, which in turn has been associated with improved long term survival [1,2]. For the first decade or so, various brands or formulations of epoetin were available, including epoetin alfa (Epogen, Procrit in the US; Eprex, Erypro in Europe). A second-generation erythropoietic agent (darbepoetin alfa) was developed by incorporating an additional two glycosylation chains to the erythropoietin molecule, resulting in a product with a longer half-life and a consequent reduced dosing frequency [3].

Since 1998, there has been a rapid increase in the incidence of epoetin therapy-associated pure red cell aplasia (PRCA), largely associated with the Eprex brand of epoetin, but described with the Epogen and NeoRecormon brands of epoetin. In 2002, Casadevall et al. [4] described a new complication of epoetin therapy, that of PRCA associated with anti-erythropoietin antibodies.

To date, however, no cases of anti-erythropoietin mediated PRCA has been described with darbepoetin alfa, and indeed it has been suggested that the additional glycosylation may cause this molecule to be more resistant to antibody generation than epoetin. We describe what we believe to be the first case of antibody-mediated PRCA associated with the administration of darbepoetin alfa alone.

Case

A 58-year-old man started haemodialysis in March 2003 for end-stage renal failure associated with chronic glomerulosclerosis. His anaemia was satisfactorily managed with darbepoetin alfa alone, and he had no exposure to any other recombinant human erythropoietin at any stage of his treatment. He was started initially on 40 mg of darbepoetin alfa, administered weekly as a subcutaneous injection. His haemoglobin began to fall in May 2004 with satisfactory haematocrits, and hence his dose of darbepoetin alfa was increased to 60 and later to 120 mg once a week with no significant response. About the same time, he developed dysphagia and significant gastrointestinal blood loss. A barium swallow showed mild gastroesophageal reflux only. Endoscopic examination of his upper gastrointestinal tract showed a small benign ulcer high in the body of the stomach along with some duodenitis. A colonoscopy revealed a 5 mm pedunculated polyp (tubular adenoma) in the sigmoid colon.

While investigating his gastrointestinal tract, his haemoglobin deteriorated disproportionately (see Figure 1), associated with a fall in reticulocyte count. His white cell and platelet counts were both normal (Table 1). Bone marrow examination in February 2005 confirmed the diagnosis of PRCA with complete absence of erythroid precursor cells. Immunophenotyping of bone marrow showed normal myeloid and lymphoid subsets. Cytogenetic analysis showed a normal male karyotype. Anti-erythropoietin antibodies were tested in February 2005 using a radio-immune precipitation assay in the Department of Immunology at King’s College Hospital in London,
and were found to be strongly positive (27% binding; normal < 0.09%). The antibodies were neutralising in a bioassay performed in Prof Casadevall’s laboratory in Paris colony-forming unit erythroid (CFU-E) growth with control serum = 170 colonies; CFU-E growth with this patient’s serum = 0 colonies. Interestingly, stored sera from May and August 2004 before the onset of reticulocytopenia also demonstrated the presence of a rising titre of neutralizing erythropoietin antibodies in the assay performed at King’s College Hospital (0.89% binding in May 2004 and 1.49% in August 2004; normal < 0.09%).

Serological tests were negative for Parvovirus B19 immunoglobulin M (IgM), cytomegalovirus IgM, but interestingly were positive for Epstein Barr virus IgM and immunoglobulin G (IgG). Epstein-Barr Virus (EBV) DNA, however, was negative, indicating that EBV IgM was false positive. Antinuclear antibodies (ANA) and C3/C4 were normal. A computed tomography (CT) scan of his chest and abdomen did not reveal any significant abnormalities, in particular there was no evidence of thymoma, lymphoma or any other tumour.

Darbepoetin alfa was discontinued and the patient started steroid therapy. There was no response in reticulocyte count after 8 weeks of steroid therapy. There was no response to a course of intravenous immunoglobulin either, at a dose of 400 mg/kg/day for 5 days. He was then started on ciclosporin with a target level of 100–150 µg/l. After 4 months of therapy, since there was no response, his treatment was changed to a combination of prednisolone and cyclophosphamide. He remained heavily transfusion-dependent and in the 13 months from December 2004 required

Table 1. Laboratory data at diagnosis

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Result</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/l)</td>
<td>77</td>
<td>125–165</td>
</tr>
<tr>
<td>WCC (×10^9/l)</td>
<td>5.8</td>
<td>4–11</td>
</tr>
<tr>
<td>Platelets (×10^9/l)</td>
<td>185</td>
<td>150–450</td>
</tr>
<tr>
<td>Retics (×10^9/l)</td>
<td>1</td>
<td>25–85</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>2832</td>
<td>22–274</td>
</tr>
<tr>
<td>B12</td>
<td>928</td>
<td>179–162</td>
</tr>
<tr>
<td>S Folate</td>
<td>3.1</td>
<td>2.7–34</td>
</tr>
<tr>
<td>LDH</td>
<td>495</td>
<td>300–650</td>
</tr>
<tr>
<td>Direct Coombs</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Ig</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Parvovirus IgM</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>CMV IgM</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>EBV IgM</td>
<td>False positive</td>
<td></td>
</tr>
<tr>
<td>EBV PCR</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

and were found to be strongly positive (27% binding; normal < 0.09%). The antibodies were neutralising in a bioassay performed in Prof Casadevall’s laboratory in Paris colony-forming unit erythroid (CFU-E) growth with control serum = 170 colonies; CFU-E growth with this patient’s serum = 0 colonies. Interestingly, stored sera from May and August 2004 before the onset of reticulocytopenia also demonstrated the presence of a rising titre of neutralizing erythropoietin antibodies in the assay performed at King’s College Hospital (0.89% binding in May 2004 and 1.49% in August 2004; normal < 0.09%).

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Fig. 1. Haematological parameters following darbepoetin and blood transfusion.
67 units of blood. During this period his serum ferritin increased from 983 ng/ml to a maximum of around 6000 ng/ml.

**Discussion**

We report the first case of PRCA with neutralizing anti-erythropoietin antibodies in a patient exposed to darbepoetin alfa (Aranesp) alone. The patient developed this condition 21 months after being on darbepoetin, having never been exposed to any other erythropoietin preparation. Anti-erythropoietin antibodies have been shown to inhibit the binding of erythropoietin to its receptor and block the differentiation of erythroid progenitors *in vitro* [5].

PRCA is the most serious adverse event to be reported following recombinant erythropoietin administration. However, the global incidence of antibody-positive PRCA is low [6].

Between 2001 and 2003, the estimated exposure-adjusted incidence of antibody-positive PRCA was highest with Eprex without human serum albumin and lowest with Epogen (Amgen), both formulations of epoetin alfa [7]. No cases of antibody-mediated PRCA have been previously reported with the sole administration of darbepoetin alfa.

For the first time, we were able to show a phase of diminished responsiveness with falling reticulocyte count and rising anti-erythropoietin antibodies to exogenous erythropoietin before the onset of reticulocytopenia. We were able to demonstrate the presence of anti-erythropoietin antibodies 7 months before the onset of reticulocytopenia. In the period between May and December 2004, although the reticulocyte count was in the reference range, there was a sharp fall in the count within the range associated with rising antibody titres. Therefore, we propose that a diminished responsiveness to erythropoietin with a sharp fall in reticulocyte count, in the absence of other causes of anaemia, justifies screening patients for anti-erythropoietin antibodies. Early detection will help to discontinue the drug and may facilitate early recovery.

Interestingly, the initial viral serological investigations in this case gave a positive result for EBV IgM and IgG. Stored blood samples going back to May 2004 tested positive for EBV IgM, which suggested that the EBV IgM was a false positive result and this was confirmed by a negative EBV polymerase chain reaction result. The presence of neutralizing anti-erythropoietin antibodies and the absence of other associated conditions make epoetin the most likely cause of the PRCA.

This case demonstrates the potential multifactorial nature of anaemia in dialysis patients and emphasizes the importance of investigating for antibody-mediated PRCA in patients receiving any erythropoietic agent, including darbepoetin alfa.

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**References**


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