CASE REPORT

Acquired Pure Red Cell Aplasia in patients with plasma cell neoplasm and long term remission with bortezomib therapy

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Abstract

We report two cases of Pure Red Cell Aplasia (PRCA) associated with plasma cell dyscrasia. The first case is of an elderly woman with PRCA and light chain disease (LCD)/multiple myeloma (MM). She received multiple blood transfusions and was refractory to conventional therapies. Subsequent bortezomib therapy resulted in symptomatic improvement and normalization of her bone marrow and transfusion independence. This unique case of LCD/MM and PRCA treated with bortezomib induced long lasting remission of myeloma and resulted in reversal of PRCA and hence transfusion independence. This novel therapy is useful in elderly patients even with a high tumour burden of multiple myeloma.

PRCA can also be associated in patients with low tumour bulk plasma cell disorders such as Monoclonal Gammopathy of Uncertain Significance (MGUS) as evidenced by the second case. Combination of prednisolone and oral cyclophosphamide therapies controlled her anemia. Hence, a tailored therapy approach is recommended. Furthermore, patients with PRCA would benefit from thorough investigation to exclude plasma cell disorders.

Key words

Light Chain Disease, Multiple myeloma, Pathogenesis, Pure red cell aplasia, Bortezomib

1 Introduction

Anemia is a common finding in patients with MM and is usually contributed to by renal impairment, systemic illnesses, marrow infiltration and hemolytic anemia. However, Pure Red Cell Aplasia (PRCA) has only rarely been reported in patients with MM, with only a few case reports documented in English literature [¹, ²]. Light Chain Disease (LCD) is a variant of multiple myeloma in which the malignant population of marrow cells produce free monoclonal light chains but no associated heavy chain or complete immunoglobulin. LCD comprises about 18% of multiple myeloma patients [³]. We report a unique case of PRCA associated with LCD/MM (lambda light chain disease) and successfully treated with bortezomib, a proteasome inhibitor. An additional case of PRCA associated with Monoclonal Gammopathy of Uncertain Significance (MGUS) is also discussed.
2 Case presentation

Patient 1: A 77 year old woman presented with symptoms of anemia, history of weight loss, back ache and arthralgia in April 2004. She had a background history of hypertension and osteoarthritis. There was no history of gastrointestinal disorders or bleeding, previous malignancies or autoimmune disorders, or evidence of neuropathy. Initial investigations showed hemoglobin (Hb) 80 g/L, white blood count (WBC) of $6.1 \times 10^9$/L, platelets $247 \times 10^9$/L and erythrocyte sedimentation rate (ESR) of 18 mm/ hour and reticulocyte count of 0.001%. Other investigations showed normal renal function, liver function and serum calcium levels. Anti-nuclear antibodies (ANA), extractable nuclear antigens (ENA), and rheumatoid factor were negative. Serum electrophoretogram (EPG) revealed paraproteinemia of 3 g/L (IgG lambda) with associated immunoperoxidase. Beta 2 microglobulin (B2M) was elevated at 4.5 mg/L. 24 hour Bence Jones protein (BJP) showed excretion of 11.5 grams/day, predominantly lambda light chain. Serum Light Chain (SLC), lambda, was elevated at 5986 mg/L (normal range: 5.7 - 26.3) and kappa/lambda ratio: 0.00088. Bone marrow biopsy revealed evidence of myeloma with greater than 50% plasma cells and almost complete lack of erythropoiesis (see Figures 1 & 2). There was no evidence of amyloidosis including on echocardiography and Congo red staining was negative. Skeletal survey showed lytic lesions in lumbar vertebrae and the skull. Parvovirus serology was negative and CT scan of the chest and the abdomen were unremarkable. There was no evidence of thymoma or lymphadenopathy. She required blood transfusions every 3-4 weeks and was commenced on melphalan and prednisolone therapies (melphalan 8 mg daily for 4 days and prednisolone 50 mg daily for 4 days) every 4 weeks. However, she developed significant pancytopenia after four courses of therapy and she remained pancytopenic despite dose reduction. Chemotherapy was ceased after ten courses. At that time, Hb was 97 g/L, WBC $1.6 \times 10^9$/L, neutrophil $0.6 \times 10^9$/L and platelets $52 \times 10^9$/L. A follow-up bone marrow aspiration and biopsy, one year after initial prednisolone and melphalan therapies, showed persistent myeloma activity with markedly reduced erythropoiesis. 24 hour urine BJP level was 4.4 grams/day and SLC (lambda) was elevated at 2654 mg/L. She had persisting fatigue, lethargy, bone pain and remained transfusion dependent. She was commenced on thalidomide and dexamethasone therapies. At the time of therapy, the patient’s clinical parameters were: Hb 86 g/L, WBC $1.9 \times 10^9$/L, neutrophils $0.8 \times 10^9$/L and platelets $28 \times 10^9$/L. She developed significant side effects on thalidomide with marked lethargy, confusion, dizziness and peripheral neuropathy and hence the therapy was suspended after four months. She was commenced on zoledronic acid infusion to reduce skeletal events and pain. She remained transfusion dependent with persisting reticulocytopenia and normal renal function. Subsequently, she was commenced on intravenous bortezomib in July 2005 at 1.5mg twice weekly for four weeks followed by a two week pause and the cycle was repeated. Over the next three months, she showed good response, and tolerated the therapy well apart from fatigue. Her peripheral neuropathy remained stable. BJP excretion and SLC (lambda) decreased to 0.8 g/day and 366 mg/L, respectively. Serum EPG showed a small abnormal band of paraproteinaemia. Calcium was normal at 2.13 mmol/L and serum creatinine had slightly worsened to 150mmol/L. Full blood count was significantly improved with Hb 129 g/L, WBC $5.1 \times 10^9$/L, platelets $129 \times 10^9$/L. Reticulocyte count was normal at 1.8%, ESR 8 mm/hour and Beta 2 M 3.9 mg/L. In January 2007, her EPG was normal and the follow bone marrow biopsy showed normocellular marrow with only 3% plasma cells (see Figure 3). SLC (lambda) was relatively stable at 537 mg/L.

By June 2008, creatinine had improved to 130 mmol/L, Hb 123 g/L, WBC $4.5 \times 10^9$/L, platelets $151 \times 10^9$/L, reticulocytes 0.7%. She received 36 packed red cell transfusions between April 2004 and August 2005 (see Figure 4) but did not require any further transfusions until November 2008. She did not require further anti -myeloma therapy. Five years after her initial diagnosis, she deteriorated following a respiratory illness and cardiac complications. At that time, there was evidence of MM recurrence with elevated lambda SLC at 4240 mg/L. However, other laboratory markers were satisfactory apart from anaemia and mild renal impairment (Hb 102 g/l and creatinine 140 mmol/l). She refused further treatment, and died of cardio-respiratory failure a month later.

Patient 2: The second case of PRCA associated with MGUS followed investigation in 2006 of a 63 year old woman with a history of anemia and background history of osteoarthritis, diabetes mellitus and hypertension. Hb 85 g/L, WBC 5.4 nL, Platelets 237 nL, Reticulocyte count 0.008%, parvoviral serology ANA, ENA and RF were negative. EPG confirmed paraprotein IgG kappa of 13 g/l, Beta 2 M 2.6 mg/L, and 24 hour urine BJP did not reveal any light chain excretion, SCL...
ratio was normal at 1.5 and bone marrow biopsy showed plasmacytosis of 6% and a virtually complete lack of erythropoiesis. Other secondary causes of PRCA were excluded. Despite ceasing all nonessential therapies, she remained transfusion dependent. She eventually responded to a combination of oral cyclophosphamide and prednisolone therapies. She remained transfusion independent over a 6 year period and progressive bone marrow biopsies showed no evolution of MGUS to MM or lymphoma or amyloidosis over the past 6 years. PRCA recurred in 2010, requiring another course of prednisolone therapy, but the patient has not required anti myeloma therapy, to date.

Figure 1. Photomicrograph of bone marrow aspirate showing hyper cellular marrow with infiltration by plasma cells (>50%), a few binucleate forms also seen, and virtually absent erythropoiesis. (H&E, X40)

Figure 2. Photomicrograph showing plasma cells infiltration with reduced hemopoietic reserves and virtually absent erythroid activity (diffuse positive lambda staining was demonstrated on immunechemical staining). (IHC, X20)

To test the capacity of bone marrow cells from this patient to form colonies in the erythroid lineage, $10^5$ marrow mononuclear cells (MMNC) were suspended in the culture medium. $10^5$ MMNC suspended in standard medium containing 1 mL a-medium containing final concentrations of 1.2% methylcellulose, 1% bovine serum albumin, mol & beta mercaptoethanol, 30% fetal calf serum (FCS), 1 U/mL recombinant human erythropoietin and 2.5% human lymphocyte conditioned medium. Cultures were incubated at 37°C in 5% CO2 and erythroid colonies (from colony forming unit-erythroid [CFU-E] and erythroid bursts (from burst forming unit-erythroid [BFU-E] were enumerated on
days 7 and 14, respectively. Granulocyte macrophage (GM) colonies (from CFU-GM) were quantified on day 14 on the same plates to control for any technical issues. All studies were performed in triplicate. Co-culture studies were performed to detect humoral or T cell factors suppressing erythroid progenitor growth. Normal in vitro growth was defined as greater than 30 BFU-E and greater than 40 CFU-E frequencies per 105 MMNC. Control BM came from a patient who was in hematological remission of a disease, generating 30-40 colonies per 105 cells plated in the same culture condition.

![Photomicrograph of the repeat bone marrow aspirate showing normocellular marrow with only 3% plasma cells and almost normal erythropoiesis two years post bortezomib therapy (H&E X40)](image)

**Figure 3.** Photomicrograph of the repeat bone marrow aspirate showing normocellular marrow with only 3% plasma cells and almost normal erythropoiesis two years post bortezomib therapy (H&E X40)

![Graph showing haemoglobin and reticulocyte counts over a 3 year period for Patient 1](image)

**Figure 4.** Hemoglobin and reticulocyte response measured in Patient 1 over a four year period

![Graph showing erythropoietin/hemoglobin levels for patient 2, during relapse (week 1) and remission (week 4) of PRCA](image)

**Figure 5.** Erythropoietin/hemoglobin levels for patient 2, during relapse (week 1) and remission (week 4) of PRCA
Cell culture studies showed reduced BFU-E colony formation when the patient relapsed in 2010 at $60 \times 10^5$, and increased to $125 \times 10^5$ on bone marrow cultures when patient was in remission, post therapy. Although cell growth was not reduced compared to control growth (40 colonies BFU-E), there was a doubling of BFU-E on culture studies ($\rho$ value 0.0001, on single tailed paired T test). CFU-E growth was $> 40 \times 10^5$ MMNC during relapse and $> 40 \times 10^5$ when in remission, hence maturation arrest between BFU-E and CFU-E differentiation. CD34 cell plated ($10^5$) with patient plasma revealed an average of four erythroid colonies during relapse, compared with an average of two colonies during remission, perhaps reflecting much greater erythropoietin levels whilst markedly anemic during relapse (see Table 1). Plasma erythropoietin levels were noted to be seven times the baseline during relapse (see Figure 5).

Table 1. Summary of hematologic parameters pre and post therapy

| Parameters                  | Response to therapy | Before | After |
|-----------------------------|---------------------|--------|-------|
|                            |                     | Patient 1 | Patient 2 | Patient 1 | Patient 2 |
|                             |                     | 80 | 86 | 129 | 126 |
| Hemoglobin                  | 0.001%              | 0.008% | 1.8% | 2.1% |
| Reticulocyte count          | 36                  | 32     | 0     | 0     |
| Paraprotein                 | 3g/l (IgG Lambda)   | 13g/l (IgG Kappa) | 1g/l (IgG Lambda) | 11g/l (IgG Kappa) |
| Serum Light Chains          | 5986mg/l            | 28mg/l | 537mg/l | 32mg/l |
| BFU-E                       | NR*                 | 60 (triplicate 58, 56, 66) | NR* | 125 (triplicate 120, 122, 133) ($\rho$ value 0.0001, on single tailed paired T test). |
| CFU-E                       | NR*                 | >40    | NR*   | >40   |
| Number of Erythroid Colonies* | NR*               | 4 (triplicate 4, 5, 3) | NR* | 2 (triplicate 2, 2, 2) |

*NR is no reliable result due to poor control growth
+Erythroid colonies observed when patient plasma added to CD34 cells plated

3 Discussion

Chronic acquired PRCA is a rare hematologic disorder characterized by selective inhibition of red cell precursors in the bone marrow. Causes of acquired PRCA include thymoma, lymphoproliferative disorders (lymphomas, chronic lymphocytic leukemia and other chronic leukemias), autoimmune disorders, medications and viral infection such as human B19 parvovirus. There are only a few published reports of PRCA associated with myeloma and other plasma cell disorders including MGUS, Waldenstrom’s Macroglobulinemia and amyloidosis [4-9].

The acute self-limited form of PRCA is secondary to virus and drug-induced impairment of erythroid progenitor cells. The acquired chronic form PRCA, inducing damage to erythroid progenitors or precursor cells, appears to be immune and T-cell mediated. In both the acute and acquired chronic forms of PRCA, the affected cells are progenitors that have differentiated from stem cells and can express erythropoietin (EPO) receptors [5, 8-10]. Thus, unlike in congenital PRCA, stem cells are not usually the targets in the acute and acquired forms of PRCA. However, in a large portion of patients the cause of PRCA remains elusive and the pathogenesis remains unclear.

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We report two cases of PRCA associated with LCD/myeloma and MGUS. The association of PRCA with MM is rare with only a few cases reported in the literature [1]. There is evidence that paraproteinemia may have a role in PRCA [2, 3, 7]. The pathogenesis of PRCA in patients with plasma cell disorders is thought to be via the inhibition of erythropoiesis by abnormal immunoglobulins produced by myeloma cells with reduction in CFU-E and BFU-E, suggesting inhibition of erythroid colonies by the monoclonal protein [3-5, 10, 11]. Elsewhere, IgG antibodies against erythroblasts, post-infectious antibodies cross reacting with erythroid precursors, or anti-erythropoietin antibodies have been suggested as playing a role in PRCA pathogenesis [12]. Our bone marrow culture studies have demonstrated serum from patient with PRCA, which is considered inhibitory for erythroblasts and responsible for the suppression of the erythrocyte colonies. In the first case presented, lambda light chain disease appears to be responsible for red cell aplasia. PRCA may also result from other mechanisms, including T-cell mediated suppression of erythroid precursors as seen in patients with chronic lymphocytic leukemia (CLL) or suppression by parvovirus infection [7, 8]. It is probable that in our patient with LCD, erythropoiesis was suppressed by monoclonal IgG lambda or SLC lambda, secreted by abnormal myeloma cells improvement of PRCA following suppression of myeloma activity supports this concept. Despite immunosuppression therapies, her disease and PRCA remained refractory until adequate disease control was achieved with bortezomib. Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome which prevents targeted proteolysis, resulting in disruption of normal homeostatic mechanisms, leading to cell death [13]. It has been shown that bortezomib functions in myeloma via the inhibition of the breakdown of inhibitory kappa B (IκB) and consequently stabilization of the nuclear factor kappa B (NFκB) complex. In turn, this results in inactivation of multiple downstream pathways known to be important in myeloma cell signaling [14]. Multiple other mechanisms for its efficacy have also been highlighted [15-17]. Tumour cells appear to be more sensitive to the effects of proteasome inhibition than normal cells due to a loss of checkpoint mechanisms occurring during tumourigenesis. This implies that normal cells can usually recover as the inhibition is transient and reversible. Consequently, this novel therapy is useful in elderly patients particularly with poor bone marrow reserve and with limited cytotoxic effects compared to conventional immunosuppressive therapies.

Cell culture studies performed in the second case presented, showed reduced BFU-E colony formation when the patient relapsed in 2010 at $60 \times 10^5$, and increased to $125 \times 10^5$ on bone marrow cultures when patient was in remission, post therapy. Although cell growth was not reduced compared to control growth (40 colonies BFU-E), there was a doubling of BFU-E on culture studies which was statistically significant ($p$ value 0.0001, on single tailed paired T test). CFU-E growth was >40 × 10^5 MMNC during relapse and > 40 × 10^5 when in remission, hence maturation arrest between BFU-E and CFU-E differentiation. This is suggestive of relative suppression of BFU-E during relapse of PRCA, most likely related to humoral factors.

MMNC late erythroid cell growth with patient plasma showed an inversely proportional relationship with the degree of anaemia, suggesting that there are additional plasma factors either promoting or suppressing erythropoiesis in these patients and that pathogenesis of PRCA is not a stem cell related anomaly in these patients (see Figure 5). BFU-E maturation in vitro has also been found to be an excellent predictor of clinical response [11] as was the case in our patient. Plasma erythropoietin levels were noted to be seven times the base line during relapse compared to levels during remission (see Figure 5). Hence it is unlikely that erythropoietin-specific antibodies were responsible for PRCA and patient plasma factors contributed by the disease may have played a role in the pathogenesis of PRCA in this patient. Previous studies have shown that factors in the patients’ sera that inhibited erythropoiesis in vivo and in vitro were followed by the demonstration that these inhibitors could be IgG antibodies directed against erythroblasts. These antibodies either inhibited haemoglobin synthesis or they were complement-binding and directly cytotoxic for erythroblasts in vitro. In patients who did not respond to immunosuppressive therapy, it is thought that such antibodies could specifically block differentiation of BFU-E in vitro [12].

Based on our experience, we recommend that patients with PRCA would benefit from thorough investigations for plasma cell disorders with therapy tailored to the individual needs of the patients and their tolerance to therapy. The second case presented highlights that conventional immunosuppression reverses PRCA if the therapy is well tolerated even if the
tumour bulk is low. It also highlights that PRCA can occur in patients with a low tumour bulk as seen in patients with MGUS. Although the pathogenesis if PRCA may be multifactorial, in patients with plasma cell disorders, it appears that monoclonal antibodies could block differentiation of BFU-E. PRCA can be reversed by reducing the monoclonal protein with immunosuppressive therapy and hence reduction of tumour burden. BFU-E maturation in vitro has also been found to be an excellent predictor of clinical response.

4 Conclusion
Pure Red Cell Aplasia is a rare complication in patients with multiple myeloma, with only a few published case reports in the literature; the possible mechanism of PRCA appears to be due to suppression of BFU-E or CFU-E by the abnormal monoclonal immunoglobulins as supported by various studies. As seen in the first case presented, although there was some improvement in bone marrow activity with chemotherapy and thalidomide, due to overall poor tolerance, other therapies were required. Resolution of PRCA with transfusion independence was achieved in this patient treated with bortezomib, with long lasting remission of both myeloma and PRCA, with improved bone marrow activity for nearly three years. The second case findings point to a previously under recognized pathogenic mechanism of PRCA associated with relatively low plasma cell tumour burden and demonstrates that these patients may benefit from anti myeloma treatment strategies, which perhaps should be tailored to the individual needs of the patients.

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Declaration of conflicting interest
There are no conflicts of interests.

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