Acquired Pure Red Cell Aplasia and Recombinant Erythropoietin

Abstract
Recombinant erythropoietin (rEPO)-associated immunologically driven acquired pure red cell aplasia (PRCA) is an underreported, potentially worsening clinical syndrome in the setting of treatment of anemia of chronic kidney disease. Most cases reported in world literature are related to different formulations of erythropoiesis-stimulating agents with an implication in diagnosis and management. This brief review highlights the clinical guidelines of rEPO usage in nephrology practice, the pathophysiologic mechanism of PRCA, clinical features, diagnosis, and suggested management protocols.

Keywords: Diagnosis, pure red cell aplasia, recombinant erythropoietin

Introduction
Pure red cell aplasia (PRCA) is a distinctly rare complication of recombinant erythropoietin (rEPO)/erythropoiesis stimulating agents (ESA) therapy for the management of the anemia of chronic kidney disease (CKD) with exposure adjusted incidence of 0.02 to 0.03 per 10,000 patient-years.[1] There was a surge in exposure adjusted incidence to 4.5 per 10,000 patient-years during 2002–2003 following the usage of epoetin alfa marketed outside the United States.[2] Till 2019, only six rEPO-associated PRCA cases have been reported from India.[3,4]

Anemia in CKD – a nephrologist’s perspective
CKD is defined as an abnormality of kidney structure or function lasting for more than 3 months with health implications. Normocytic normochromic anemia is observed as early as stage III CKD and is almost universal by stage IV; and the severity of anemia increases with declining renal function.[9,10] As per recent updated Kidney Disease Improving Global Outcomes (KDIGO) guidelines, renal anemia should be diagnosed at hemoglobin (Hb) of less than 13.0 g/dL in males and less than 12.0 g/dL in females; and this is attributed primarily to the relative EPO deficiency and to some extent associated with additional factors such as diminished red cell survival, chronic inflammation, iron deficiency, bleeding diathesis, and hyperparathyroidism.[9-11] The prevalence of anemia increases from 1% among patients with an estimated GFR (eGFR) of 60 mL/min/1.73 m² to 9% at an eGFR of 30 mL/min/1.73 m²; and to 33 to 67% at an eGFR of 15 mL/min/1.73 m².[12] It contributes considerably to reduced quality of life of patients with CKD and has been associated with several adverse clinical outcomes like fatigue, diminished exercise tolerance, angina, heart failure, decreased cognition, and impaired host defense against infection. The availability of rEPO has been one of the most significant advances in the care of CKD patients. The use of rEPO has significantly reduced the need for regular blood transfusion in CKD patients, thus dramatically reducing the transfusion-related complications.[13]

Recombinant erythropoietin
The first commercially available rEPO was epoetin alfa, approved by US food and drug administration in 1989, which is produced by recombinant DNA technology in massive cell culture. The second rEPO developed was darbepoeitin alfa and the third one was methoxy polyethylene glycol-epoetin beta, which has significantly increased serum half-life. The different commercially available rEPOs used in clinical practice are presented in Table 1 and their clinical dosing schedule is presented in Table 2.[12-15]
The Hb level at which rEPO should be initiated remains controversial. Based on the finding of famous studies like Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) trial,[12] Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) trial,[13] and Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) trial,[14] KDIGO guidelines recommend the use of rEPO to prevent the Hb level in dialysis patients from falling below 9.0 g/dL. However, individualization of therapy is reasonable as some patients may have an improvement in the quality of life with a higher Hb level. KDIGO guidelines also suggest not to start rEPO in non-dialysis CKD patients with a Hb concentration of more than 10.0 g/dL.[9] Within 3 to 4 days of initiation of therapy, an increase in reticulocyte count is noted; and within 1 to 2 weeks, there is a significant rise in Hb concentration in the order of 0.25 to 0.5 g/dL/week. Over the course of 1-month therapy, a significant increase of 1 to 2 g/dL in Hb concentration is usually achieved. If a patient fails to respond satisfactorily then the dose of rEPO is increased in the stepwise upward titration of 25 to 50% monthly until a good response is achieved.[11-13] All acquired knowledge on rEPO therapy in patients with CKD indicated clear benefit when Hb is less than 10 g/dL whereas there is an increased risk of stroke, arteriovenous fistula (AVF)-related complications when Hb target exceeds 13 g/dL. In CKD patients as Hb concentration exceeds 11 g/dL and approaches 13 g/dL, the potential benefits diminish, and risks increase.[11]

Clinical presentation

Bennett and colleagues reviewed the follow-up outcome data of 170 out of 191 rEPO-associated PRCA cases associated with CKD as reported by the US Food and Drug Administration Research on Adverse Drug Events and Reports (RADAR) project.[17] The majority of the cases (>50%) occurred in the elderly age group (mean age; 62 ± 17 years) with a male preponderance (66% vs. 34% females). About 169 cases had received rEPO via a SC route, whereas one epoetin alfa-treated patient received via the IV route.

Epidemiology of rEPO associated PRCA

Following the introduction of epoetin alfa formulation (in 1988) for the management of the anemia of CKD, three cases of PRCA were reported in the subsequent 10 years.[1,2] In 1998, to reduce the risk of possible transmission of a variant form of Creutzfeldt-Jakob disease via human serum albumin (HSA) in the European countries, this formulation was changed to HSA free formulations rich in excipients polysorbate 80 and glycine.[16] Besides, both clinical and economic considerations led to a shift in the administration of epoetin from the intravenous (IV) route to the subcutaneous (SC) route in many countries. The subsequent 5 years witnessed nearly 10-fold increased incidence of PRCA cases following the usage of reformulated formulations of a specific brand in comparison to the epoetin beta formulation and the epoetin alfa formulation Epogen. By 2005, nearly 200 cases of PRCA were reported among CKD patients receiving these formulations subcutaneously, worldwide; European countries accounted for nearly 60% of all related cases to a particular brand, and only four cases were reported from the United States secondary to usage of epoetin alfa. Sporadic cases of rEPO-associated PRCA were also reported from the Indian subcontinent over the last decade following administration of such analogs; mostly through SC route and rarely through IV route. rEPOs implicated were darbepoetin alfa, and epoetin alfa.[3-8]
The mean duration of usage of before the development of PRCA was 9 months for epoetin alfa, 18 months for epoetin beta, and 24 months for other epoetin alfa. The hematologic recovery (following the withdrawal of rEPO) with and without immunosuppressive therapy was highly significant (57% vs. 2%, respectively, \( P < 0.001 \)); and nearly 95% had complete recovery following renal transplantation \( (n = 19 \) cases). Among 34 patients who received epoetin alfa after the onset of PRCA, 56% regained epoetin alfa responsiveness. Nearly 90% of the patients who did not have detectable anti-EPO antibodies in their serum achieved hematologic recovery following rechallenge with rEPO.\(^\text{[17]}\) The six cases reported from India (2005 to 2019) occurred in five males and one female with end-stage renal disease; and among five of them where detailed follow-up data were available, three had successful hematological recovery following immunosuppressive therapy and/or renal transplantation;\(^\text{[3-8]}\) one had a dramatic response to rituximab therapy\(^\text{[8]}\); whereas rechallenge with a different rEPO (darbopoetin-\(\alpha\)) along with immunosuppressive therapy lead to a recovery in another individual (mean duration of follow-up 6 months, the longest being 10 years).\(^\text{[7]}\)

**Pathophysiologic mechanism**

The pathophysiologic mechanism linked to rEPO-associated PRCA is complex and seems to be multifactorial [Figure 1]. As hypothesized by Macdougall et al., the central event seems to be a breach in B-cell immunological tolerance resulting in increased antigen recognition by autoreactive T-cells and subsequent B-cell-mediated antibody response in genetically susceptible (HLA-DRB1*9 positive) individuals.\(^\text{[1,18]}\) The HSA used in previous formulations was less immunogenic due to its stabilizing effect on proteins; whereas polysorbate 80 and tungsten (in vials) in newer formulations help conformational changes of protein moiety causing more aggregation; hence more antigenicity.\(^\text{[19]}\) It is hypothesized that the periodicity of polysorbate 80 micelles containing protein or their aggregates breaks the self-tolerance by its resemblance to the repeated self-epitope structure of viral capsids that is capable of directly activating B-cells.\(^\text{[20]}\) Another explanation proposed that organic leachates from the uncoated rubber stoppers of pre-filled syringes act as adjuvants for T-cell-mediated activation of the anti-EPO immune response; though this theory has not been substantiated in experimental models.\(^\text{[21]}\) The SC route of administration, compared to the IV route has been postulated to be more immunogenic as has been published in the literature. This is due to the slower rate of absorption and hence increased antigenic recognition and presentation by cutaneous antigen-presenting cells (Langerhan cells) to autoreactive T-cells. Besides, the SC route of administration offers self-administration and hence inappropriate handling at home. The SC route of administration is also linked to the longer duration of treatment as most of the previously published series have reported the occurrence of PRCA following a median duration gap of 9 months (range: 3 months to 67 months).\(^\text{[22]}\) The duration of treatment has been proposed to explain, at least in part, why no cases of PRCA have been reported in patients with cancer receiving rEPOs for chemotherapy-induced anemia, who typically receive rEPO treatment for a much shorter period than patients with renal anemia. Such patients may also have a compromised immune response because of the myelosuppressive effects of chemotherapy.\(^\text{[23]}\) Patient-related factors such as advanced age, concurrent infections, intermittent illness, adjuvant therapies, comorbidities, and immune status of the individuals all might contribute to immune dysregulation as well.\(^\text{[2]}\)

*Figure 1: The proposed pathophysiologic mechanism in antibody-mediated rEPO associated PRCA (adopted from Macdougall et al.)\(^\text{[19]}\).* HAS; human serum albumin, APC; antigen-presenting cells, EPO; erythropoietin, rEPO; recombinant erythropoietin
Diagnosis and Management

The diagnosis of rEPO induced PRCA in CKD subjects should be made when there is resistance to rEPO therapy as evidenced by the rapid decline in Hb levels to 5 to 6 g/dL or transfusion dependence. This is corroborated with reticulocytopenia (<10 × 10^6/L) in presence of normocellular bone marrow (BM) showing normal myeloid and megakaryocytic maturation and distribution.[16] Although BM examination in such cases shows the absence or near-total absence of erythroid precursors, the diagnosis rests upon demonstration of anti-EPO neutralizing antibodies (NAbs); either by binding antibody assays such as radioimmunoprecipitation (RIP) assays, enzyme-linked immunosorbent assays (ELISAs) and the surface plasmon resonance methods (BIAcore immunoassay). Samples that are positive for the presence of anti-EPO antibodies are then tested for the presence of EPO-NAbs using a cell-based bioassay.[24]

Both antibody-dependent assays and bioassays have their advantages and disadvantages.[25] Both ELISA and RIP assays are commonly used, highly sensitive, and cost-effective methods; the former uses plastic wells as the solid support for Ab testing whereas the latter measures the radiolabeled antigen binding to a coated Ab in a fluid state. ELISA has the disadvantages of giving a false negative result in the case of low-affinity Abs as well as high background readings. RIP assay has the issue of rapid decay of radiolabeled antigens; and immunoglobin M (IgM) Abs may go undetected. Both RIP and ELISA-based assays are prone to false-positive results secondary to interference with IgM rheumatoid factor, naturally occurring antibodies (ELISA), or nonspecific background noise. Other than high cost and lack of expertise issues, BIA core immunoassay offers the advantage of detecting both low and high-affinity Abs and isotypes using real-time binding and competitive analysis. Bioassays though give a quantitative measurement of NAbs are moderately sensitive, time-consuming (days) due to different cell lines used. Similar to ELISA and RIP, this is also prone to false-positive results secondary to interference with serum neutralizing factors arising from preexisting morbidities. Therefore, bioassay results should be interpreted in the context of antibody assay results.[25] However, all these testing facilities are either not available or not routinely performed in most of the centers rendering the EPO-PRCA underreported/underdiagnosed.

Ab-mediated PRCA is rarely self-limiting and, therefore, requires immediate stoppage or withdrawal of rEPO-based therapy followed by correction of anemia by red cell transfusions.[26-28] The definitive way of management is the initiation of immunosuppressive therapy with corticosteroids alone (starting dose; 0.5 to 1.0 mg/kg/day) or in combination with cyclosporine A [4-8 ± 1·2 mg/kg with a range of 2-9 to 7·6 mg/kg; most often 200 to 300 mg/day] or cyclophosphamide (200 mg/day).[29] The patients who are good responders are expected to be benefited within a span of 3 to 4 months (measured by an increase in reticulocyte count or decrease transfusion requirement). Renal transplantation is the most effective way of management of rEPO-associated PRCA in CKD subjects with near-total recovery in most of the cases. Transplanted patients also receive immunosuppressive therapy as part of their anti-rejection protocol, and this is unclear whether the success of this strategy is related to this therapy or to the transplant itself.[26] The real challenge for nephrologists is whether rEPO therapy can be re-started in patients who have recovered from PRCA with the disappearance of NAbs. Rechallenge with a rEPO can cause a relapse and may induce systemic reactions. Successful rechallenge has been observed in isolated cases, although this should be carefully considered; and should preferentially use IV administration.[28-30]

Conclusion

To conclude, rEPO-associated Ab-mediated PRCA is a distinctly uncommon, yet potentially underreported complication in CKD subjects who require prolonged therapy for their management. The exact pathogenesis seems to be multifactorial; and points to a breach in the self-tolerance mechanism of humoral immunity. While renal transplantation is the most effective way of managing such cases, immunosuppressive therapy is also an option for transplant-ineligible subjects. Close coordination between clinicians/nephrologists, transplant experts, pharmaceutical industries, laboratory, and the patient is essential for a better understanding of the pathogenesis. The potential benefit of protein mimetic agents is also a promising development and requires further research and clinical trials.

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Conflicts of interest
There are no conflicts of interest.

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