

Anaemia, rHuEPO resistance, and cardiovascular disease in end-stage renal failure: links to inflammation and oxidative stress

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Abstract

End-stage renal disease (ESRD) is characterized by a high mortality rate, derived largely from cardiovascular disease (CVD). In patients with ESRD, high levels of pro-inflammatory cytokines and increased oxidative stress are common features that may contribute to malnutrition, anaemia, recombinant human erythropoietin (rHuEPO) resistance, and atherosclerosis. Inflammation predicts poor outcome in ESRD. It is multifactorial in cause and, while it may reflect the underlying CVD, the acute-phase response may also contribute to both oxidative stress and progressive vascular injury. In patients with ESRD, the acute-phase response may be influenced by a number of factors unrelated to dialysis and perhaps by the dialysis procedure itself. Inflammation and the acute-phase response interact with the haematopoietic system at several levels resulting in reduced erythropoiesis, accelerated destruction of erythrocytes, and blunting of the reactive increase in erythropoietin in response to reduced haemoglobin levels. In patients with ESRD, rHuEPO resistance has been linked with inflammation, the latter of which is often associated with a state of functional iron deficiency. Patients with ESRD are thought to have a reduced capacity to handle oxidative stress. There is recent evidence that a relationship may exist between inflammation and oxidative stress and treatment of anaemia with rHuEPO. However, iron may also generate oxidative stress. Controlled trials are needed before evidence-based recommendations for the management of inflammation-induced anaemia and resistance to rHuEPO can be defined.

Keywords: anaemia; atherosclerosis; end-stage renal disease; erythropoietin; inflammation; oxidative stress

Inflammation is a common feature of ESRD

It is well known that ~30–50% of pre-dialysis, haemodialysis (HD), and peritoneal dialysis (PD) patients have serological evidence of an activated inflammatory response with elevated (>8–10 mg/l) serum levels of C-reactive protein (CRP) [1]. The acute-phase response may be influenced by a number of non-dialysis related factors such as age, race, gender, and residual renal function [2]. However, the highly skewed distribution of commonly used inflammatory markers, such as CRP and interleukin 6 (IL-6), suggest that patient-specific processes may also contribute to inflammation in patients with end-stage renal disease (ESRD). Examples of these patient-specific processes include clotted access grafts [3] and persistent infections, such as *Chlamydia pneumoniae* [4] and dental infections [5]. Moreover, increased levels of pro-inflammatory cytokines have been reported in HD patients suggesting that the dialysis procedure, with its extracorporeal circulation of blood, may itself cause inflammation [6,7]. Non-biocompatible dialysis membranes [8], non-sterile dialysate [9], and back-leak of dialysate across the dialysis membrane [10] have also been associated with an inflammatory reaction in HD patients.

Inflammation predicts poor outcome in ESRD

The annual cardiovascular disease (CVD) mortality rate in patients with ESRD is 10–20-fold higher than in the general population, even when adjusted for age, gender, race, and diabetes mellitus [11]. Although dyslipidaemia, hypertension, and smoking are prevalent in patients with ESRD, these traditional risk factors may not adequately account for the high prevalence and incidence of CVD [12]. It has been postulated that non-traditional risk factors, such as oxidative stress and inflammation, may contribute to the high prevalence of CVD in this patient group

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[1,13]. Indeed, a strong association between elevated CRP and increased mortality has been reported in HD [14,15] and PD [16] patients. As CRP has been shown to be an independent predictor of the number of atherosclerotic plaques in the carotid arteries of dialysis patients [17] and strongly related to surrogate markers of atherosclerosis in pre-dialysis patients [18], the acute-phase response may play a significant role in the accelerated atherogenesis of ESRD. CRP is a precise objective index of inflammatory activity and reflects the generation of pro-inflammatory cytokines, especially IL-6 activity. As a consequence, elevated serum IL-6 levels have been associated with increased mortality in HD [19] and PD (Pecoits-Filho R, Bárány B, Lindholm B, Heimbürger O, Stenvinkel P, 2002, submitted for publication) patients, and can predict accelerated atherosclerosis during dialysis treatment [20]. It is also worth noting that chronic inflammation and elevated levels of pro-inflammatory cytokines might be an important cause of wasting in patients with ESRD [21].

Inflammation and increased oxidative stress

Patients with ESRD are thought to have reduced capacity to handle oxidative stress, as indicated by increased lipid peroxidation [22] and decreased levels of antioxidants such as glutathione, vitamin E and C, and superoxide dismutase [23,24]. In a recent study of patients with peripheral vascular disease, serum vitamin C concentrations were found to be inversely related to CRP [25]. Also, malnourished patients with ESRD have evidence of increased oxidative stress [26] that may indirectly suggest a relationship between inflammation and increased oxidative stress. Plasma proteins, as well as lipoproteins, are important targets of oxidation and serum albumin has been recognized as the major plasma protein target of oxidant stress in ESRD [27]. As albumin is very vulnerable to the effects of inflammation, it could be hypothesized that oxidative stress and its related biological effects have a pathological relevance in many disease states associated with inflammation. Indeed, a recent study by Heitzer *et al.* [28] demonstrated that increased vascular oxidative stress predicts the risk of cardiovascular events in patients with coronary heart disease. This finding supports the concept that oxidative stress may be a significant risk factor for accelerated atherosclerosis, a process strongly associated with inflammation [29]. A link between inflammation and oxidative stress was first suggested by Memon *et al.* [30] who demonstrated that low-density lipoprotein (LDL) from animals treated with bacterial lipopolysaccharide (LPS) was more susceptible to *ex vivo* copper-dependent oxidation and modification to an atherogenic form, than LDL isolated from saline-treated animals. Recent results suggest that inflammation causes increased oxidative stress in patients with ESRD. Nguyen-Khoa *et al.* [31] suggested that the

presence of inflammation and the duration of dialysis are the most important determinants of oxidative stress in HD patients. Moreover, an association between F₂-isoprostanes (a surrogate marker for oxidative stress) and CRP levels has been reported in HD patients [22] and in patients with advanced ESRD [32].

Inflammation and anaemia

Inflammation and the acute-phase response interact with the haematopoietic system at several levels. During the early period of the acute-phase response, haemoglobin (Hb) concentration often drops rapidly. This is due to the accelerated destruction of erythrocytes by inflammatory-activated reticulo-endothelial macrophages that efficiently clear the circulation of erythrocytes coated with immunoglobulins or immune complex [33]. In patients with normal renal function, an acute decrease in Hb concentration stimulates secretion of erythropoietin for 4–10 days. Importantly, this reactive increase in erythropoietin secretion is blunted by pro-inflammatory cytokines in patients undergoing acute-phase response [34].

Growth factors, including erythropoietin and several cytokines, are necessary for the normal growth and differentiation of erythroid progenitors in the bone marrow. In low concentrations, the pro-inflammatory cytokines tumour necrosis factor- α (TNF- α) and IL-1 stimulate growth of early progenitors (burst-forming units-erythroid) [35]. The erythropoiesis-suppressing effect of inflammation is mainly due to increased activity of the pro-inflammatory cytokines on these precursor cells at various stages of erythropoiesis. It is thought that this inhibitory effect on erythroid precursors is primarily due to alterations in sensitivity to erythropoietin [35]. In studies in animal models and in humans, administration of TNF- α , IL-1, or IL-6 causes hypoproliferative anaemia directly by action on erythroid progenitor cells, or indirectly by stimulation of interferon production [35–39]. Accordingly, in patients with chronic inflammatory disorders, such as rheumatoid arthritis, TNF- α blockade has been associated with an increase in Hb levels [40]. The inhibitory effect of TNF- α and IL-1 on erythropoiesis can be overcome dose-dependently by administering higher doses of recombinant human erythropoietin (rHuEPO). In patients with ESRD, rHuEPO resistance has been linked with an inflammatory response as patients with elevated CRP [41] or fibrinogen [42] levels are less responsive to rHuEPO. Gunell *et al.* [43] reported that low serum albumin and high CRP levels also predicted rHuEPO resistance both in HD and PD patients, lending additional support to the concept that the inflammatory response causes both hypoalbuminaemia and anaemia in ESRD patients.

Inflammation and iron metabolism

Inflammation is often associated with a state of functional iron deficiency with low serum levels of iron and transferrin [44,45]. In these cases, delivery of iron from reticuloendothelial cells to haematopoietic cells is inhibited or blocked [44,45]. During inflammation, lactoferrin-bound iron is taken up by activated macrophages that express specific lactoferrin receptors. This causes iron deprivation in the erythroid precursors, which fail to express lactoferrin receptors [35,36]. These inflammatory-induced effects on iron metabolism are thought to comprise part of the host-defence mechanisms against bacterial and viral infection [46]. Serum ferritin, which acts as an acute-phase protein, increases 2–4-fold in response to inflammation [36]. In addition, both IL-1 and TNF- α cause a direct increase in ferritin synthesis by acting on gene transcription [47,48]. However, cytokines may also induce ferritin synthesis indirectly by increasing iron uptake into hepatocytes [36]. This increase in ferritin synthesis by hepatocytes and reticuloendothelial cells underlies the increase in the iron storage pool during inflammation. Also of relevance is that inflammation affects mucosal uptake and transfer of iron, thus reducing absorption of oral iron and further contributing to anaemia [49]. Finally, pro-inflammatory cytokines, such as IL-6 and TNF- α , have been found to induce intestinal bleeding in rats [50].

Is anaemia related to increased CVD mortality in ESRD?

Observational studies have associated anaemia with increased CVD and all-cause mortality both in ESRD patients [51] and in the general population [52]. Although strong associations between raised levels of pro-inflammatory cytokines and poor outcome in patients with ESRD are documented [19,53, Pecoits-Filho R, Bárány B, Lindholm B, Heimbürger O, Stenvinkel P, 2002, submitted for publication], the independent role of anaemia in this scenario has yet to be defined. Lowrie [54] postulated that anaemia and malnutrition share the inflammatory response as a common cause. In accordance, we have found significant associations between elevated CRP (≥ 10 mg/l) and a poor outcome during dialysis treatment, as well as low (≤ 102 g/l) Hb levels in patients with ESRD close to start of regular dialysis treatment (Figure 1). However, so far, only one interventional study has been designed to evaluate the effect of rHuEPO correction of anaemia of ESRD in patients with CVD, and this failed to demonstrate a significant effect on mortality [55]. Thus, further studies are needed to elucidate the independent role of anaemia as a risk factor for increased cardiovascular mortality in patients with ESRD.

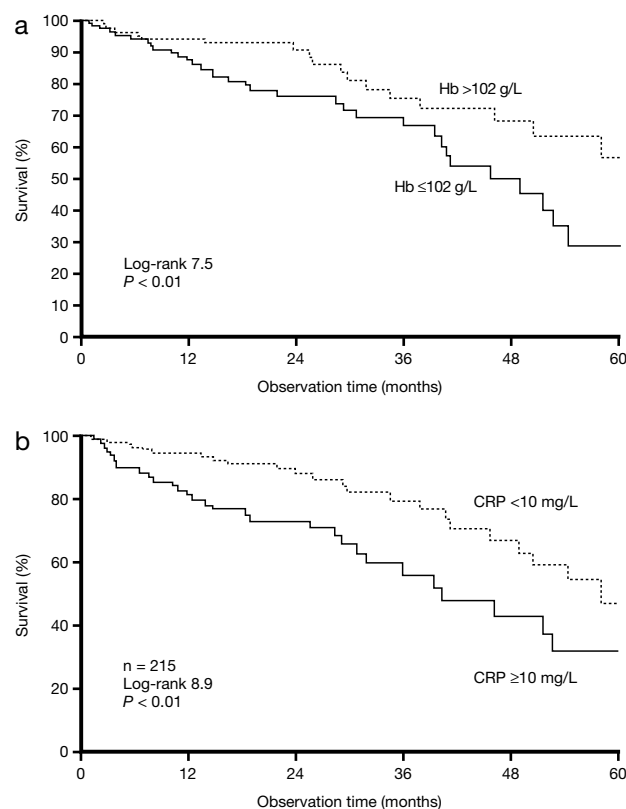


Fig. 1. Kaplan–Meier plots of the survival rate of ESRD patients starting dialysis treatment. Hb and CRP levels were measured close to the start of dialysis treatment. The patients ($n=215$) were divided into two groups according to (a) the median Hb level, that is, ≤ 102 g/l (solid line) and > 102 g/l (dashed line) and (b) the CRP level, < 10 mg/l (dashed line) and ≥ 10 mg/l (solid line), respectively. This figure represents the unadjusted overall mortality of dialysis patients and not only mortality caused by cardiovascular events. Difference between the groups in both analyses, $P < 0.01$.

Does anaemia cause increased oxidative stress?

Several recent studies have addressed the issue of whether anaemia in patients with ESRD is related to increased oxidative stress. It has been reported that peroxidation of lipids within the red blood cell (RBC) membrane may contribute to shortening RBC life span [56] and that anaemia is associated with higher plasma concentrations of lipid peroxidation products in HD patients [57]. Moreover, lower plasma levels of a surrogate marker of oxidative stress, malondialdehyde (MDA), have been observed in rHuEPO-treated patients with higher Hb levels [58]. This finding may reflect decreased free radical generation. However, in an earlier study, no longitudinal effect on MDA levels was demonstrated after partial correction of renal anaemia with rHuEPO [56]. Why anaemia may be associated with increased oxidative stress is not fully understood, although there is speculation that factors such as hypoxia and alterations in catecholamine metabolism may play a role [58]. It is also possible

that treatment of anaemia is associated with an increased availability of glutathione and other anti-oxidants [59] as higher levels of anti-oxidant enzymes in the RBC membrane are associated with an increased number of immature RBCs [56].

The treatment of anaemia in ESRD patients with inflammation

On average, it has been estimated that the rHuEPO dose required to maintain target Hb level may be increased by 30–70% in inflamed dialysis patients (CRP > 20 mg/l) compared with those with a lower CRP concentration [41, and unpublished data from the European Survey of Anaemia Management]. Indeed, in severe systemic inflammation, the response to epoetin may be totally blunted and blood transfusion may be needed. As inflammation may be a major cause of rHuEPO resistance, it is evident that eradication of inflammatory stimuli should be a primary aim in treating inflamed and anaemic patients. However, although there is a high prevalence of inflammation in patients with ESRD, there are, as yet, no valid recommendations on how chronic inflammation should be handled in these individuals [2]. Obviously, comorbid conditions that may contribute to inflammation, such as persistent infections, chronic heart failure, and coronary heart disease should be adequately treated. Moreover, as the HD procedure *per se* may cause an inflammatory response, the benefits of using biocompatible membranes [60] and ultrapure dialysate [61] warrant further investigation. Interestingly, the use of ultrapure dialysate has been found not only to reduce various inflammatory parameters but also to improve the response to rHuEPO in HD patients [61].

As increased oxidative stress may be associated both with inflammation and anaemia, as well as rHuEPO resistance, it could be hypothesized that various anti-oxidative treatment strategies may have rHuEPO-sparing effects. As a result of its documented protective effect against lipid peroxidation, vitamin E appears to be an ideal agent to decrease oxidative stress in dialysis patients [62,63]. Indeed, in a recent clinical study high-dose vitamin E supplementation was found to reduce CVD endpoints and myocardial infarction in HD patients, although no reduction in all-cause mortality was demonstrated [64]. Vitamin E modification of the dialyzer membrane has been reported to reduce the consequences of neutrophil activation, such as release of myeloperoxidase [65]. However, to the best of our knowledge, no data are yet available on the effects of vitamin E-modified dialysis membranes on CVD morbidity and mortality. Interestingly, recent data suggest that anti-oxidative treatment strategies may also modulate the inflammatory response. First, vitamin E supplementation in non-renal patients was associated with a decrease in CRP and monocyte IL-6 level [66]. Secondly, indirect support for an anti-inflammatory effect of vitamin E (and other anti-oxidants such as vitamin C, melatonin, and glutathione) has come from

studies indicating that they may elicit an improvement in the therapeutic response to rHuEPO in dialysis patients [67–71]. Thirdly, it has been hypothesized that anti-oxidative treatment strategies prevent oxidative haemolysis of RBC membranes [72], and thus provide another potential mechanism by which anti-oxidants may improve the response to rHuEPO.

Conclusion

ESRD is characterized by a high mortality rate derived largely from CVD. In patients with ESRD, high levels of pro-inflammatory cytokines and increased oxidative stress are common features that may contribute to malnutrition, anaemia, rHuEPO resistance, and atherosclerosis by different pathogenetic mechanisms. Inflammation is multifactorial in cause and while it may reflect underlying CVD, the acute-phase response may also contribute to both oxidative stress and progressive vascular injury. Recent findings suggest that anaemia is associated with increased oxidative stress and various anti-oxidant treatment strategies have been associated both with a reduction in oxidative stress and in the required dose of rHuEPO in patients with ESRD. Conversely, there may be pro-oxidant effects from treatment of anaemia with rHuEPO and iron. Controlled trials are needed before evidence-based recommendations for the management of inflammation-induced anaemia and rHuEPO resistance can be defined. In particular, the risks and benefits of i.v. iron and the effects of various iron dosage schedules warrant further careful evaluation in prospective studies.

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