Erythropoiesis Stimulatory Agent-Resistant Anemia in Dialysis Patients: Review of Causes and Management

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Key Words

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Abstract

Despite new therapeutic options and treatment strategies, anemia still remains one of the major complications of chronic kidney disease (CKD), especially in patients undergoing chronic hemodialysis for end-stage renal disease. Successful management of anemia is a central part of patient care that may improve clinical outcomes. Although the National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF-DOQI) working group reformulated its recommendations by stating that the hemoglobin target in patients receiving erythropoiesis stimulatory agents (ESA) should generally be 11–12 g/dl, this target value can not be achieved in many of them, despite treatment with high doses of ESA. The aim of the present review is to provide an update of the recent literature on causes and possible management of ESA-resistant anemia in CKD patients.

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Introduction

The World Health Organization (WHO) defined anemia as a hemoglobin (Hb) level of less than 13.0 g/dl for adult males and postmenopausal women and less than 12.0 g/dl for premenopausal women [1]. Based on these criteria, nearly 90% of patients with a glomerular filtration rate (GFR) of less than 25–30 ml/min have anemia [2]. Despite new therapeutic options and treatment strategies, anemia remains one of the major complications of chronic kidney disease (CKD), especially in patients undergoing chronic hemodialysis (HD) [3]. The presence of anemia in these patients reduces quality of life and contributes to symptoms of advanced renal failure, such as fatigue, reduced exercise tolerance, depression and dyspnea [4]. Moreover, anemia is associated with worsening of cardiovascular morbidity and accelerated rate of kidney damage, and it is an independent predictor of mortality in CKD patients [5]. Therefore, successful management of anemia is a vital part of patient care that may potentially improve clinical outcomes.

Although the United States National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF-DOQI)

working group reformulated its recommendations by stating that the hemoglobin (Hb) target in patients receiving erythropoiesis stimulatory agents (ESA) should generally be 11–12 g/dl, this target value cannot be achieved in several of them, despite treatment with high-dose ESA [5–7]. The introduction of recombinant human erythropoietin (rHuEPO) therapy in the early 1990s led to a significant decrease in persistent anemia prevalence in CKD patients. According to the literature, 90% of renal anemia patients responded in a dose-dependent manner to rHuEPO, whereas the remaining 5–10% of patients had either a blunted or absent response to this agent, despite high-dose therapy [8].

Lower erythropoietin responsiveness is a strong, independent predictor of mortality risk. Both the inability to achieve a target hemoglobin and administration of high-dose epoetin-alpha were each significantly associated with increased risk of death, myocardial infarction, congestive heart failure or stroke [9, 10].

The aim of the present review is to provide an update of the recent literature on causes and possible management of ESA-resistant anemia in CKD patients.

Definition and Diagnosis

The NKF-DOQI defined the hyporesponsiveness to erythropoietin as the presence of at least one of the following three conditions: a significant decrease in Hb level at a constant ESA dose, a significant increase in the ESA dose requirement to preserve a certain Hb level, or a failure to raise the Hb level to greater than 11 g/dl despite an ESA dose equivalent to erythropoietin greater than 500 IU/kg/week [11].

Similarly, European guidelines also recommend consideration of ESA resistance when a patient either fails to attain the target hemoglobin concentration while receiving more than 300 IU/kg/week (20,000 IU/week) of erythropoietin or 1.5 mg/kg of darbepoetin-alfa (100 mg/week), or has a continued need for such high dosages to maintain the target [12].

The 'erythropoietin resistance index' (ERI), (rHuEPO/kg/week divided by Hb level in g), is an alternative method, considered by some as a better way to measure the degree of ESA resistance. An ERI value greater than 0.02 µg/kg/week/g Hb indicates resistance to ESA; additionally the time course of ERI may be important to demonstrate the degree of response to these agents [13].

Etiology

The main cause of ESA-resistance is iron deficiency, but rHuEPO-resistant anemia persists in some HD patients even after sufficient iron supplementation [14]. Iron deficiency may be absolute – with a ferritin concentration less than 100 mg/l – or functional – when the ferritin concentration is greater than 100 mg/l and total saturation of transferrin (TSAT) less than 20%; besides noncompliance (that should be checked when Epo is self-administrated). Other recognized causes of ESA-resistance in patients with adequate iron stores include concomitant inflammation or infection, neoplasia, chronic hemolysis, hemoglobinopathies, severe hyperparathyroidism, aluminum intoxication, vitamin B₁₂ or folate deficiencies, inadequate dialysis, myelosuppressive agents, myelodysplasia, antibody-mediated pure red cell aplasia, thyroid dysfunction, and some drugs, including RAAS blockers [6, 15–19] (fig. 1).

Cytokines and Inflammation

Recently, genetic polymorphisms for cytokine genes have been identified as playing an important role in the pathogenesis of anemia by influencing the level of corresponding cytokines [20]. A recent clinical study including 167 maintenance hemodialysis patients reported that patients with the ACE DD genotype had significantly lower ERI values compared to those with ACE II or ACE ID, independent of other traditional risk factors for anemia. Moreover, this study also revealed that the IL-1B-511CC genotype was significantly associated with lower ERI values in HD patients [21].

Inhibition of erythropoiesis by cytokines, such as tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) are also important for erythropoietin resistance [22]. In CKD patients elevated levels of IL-1, IL-6, TNF- α and CRP (suggestive of a chronic inflammatory status) have been frequently described [23, 24]. Del Vecchio et al. [25] reported that cytokine-induced inflammation suppresses bone marrow erythropoiesis in HD patients and is a possible cause of anemia. Shinzato et al. [26] found higher levels of ferritin, hs-CRP and IL-6 in 19 HD patients with rHuEPO-resistant anemia, compared to control HD patients without anemia and iron deficiency. In a randomized study of Costa et al. [19] on 50 HD patients, non-responders to ESA treatment had higher CRP, lower serum albumin levels as well as lower number of total and CD4+ lymphocytes, in comparison to responders. This suggests a relationship between resistance to rHuEPO therapy and the magnitude of the inflammatory re-

Fig. 1. Causes of ESA-resistant anemia in CKD.

sponse. Although resistance to rHuEPO therapy is associated with inflammation markers and CD4+ lymphopenia it could not be ascribed to an enhanced T cell activation state or a mediated Th1 response. More recently Kalantar-Zadeh et al. [27] confirmed the strong association between indices of EPO hyporesponsiveness and high levels of inflammatory markers in a larger cohort of 339 HD patients.

Primary and secondary neutrophil granules contain elastase and lactoferrin, respectively, which are commonly used as indirect markers of neutrophil activation in vivo. Costa et al. evaluated the neutrophil activation state in CKD, in 63 HD patients under rHuEPO treatment (32 responders and 31 nonresponders to rHuEPO therapy). Compared with controls, CKD patients presented with significantly higher CRP and neutrophil and elastase lev-

els. Moreover, nonresponders to the rHuEPO therapy developed statistically significantly higher elastase plasma levels than rHuEPO therapy responders, which could be related to the rise in neutrophils and be part of the enhanced inflammatory process found in these patients. On the other hand, plasma levels of lactoferrin and the lactoferrin/neutrophil ratio did not differ between groups. Therefore, elastase, but not lactoferrin may be a better marker of resistance to rHuEPO therapy in CKD patients under hemodialysis [28].

Hepcidin

Hepcidin, a small cysteine-rich polypeptide, is a mediator of innate immunity, mainly produced by hepatocytes and found to be a key regulator of iron homeostasis [29]. Hepcidin binds to ferroportin, a cellular iron ex-

porter, and suppresses extracellular release of iron by decreasing ferroportin. The production of hepcidin is enhanced by iron overload, inflammation and IL-1 and IL-6 [30–32]. Ferroportin is highly expressed in macrophages of the reticuloendothelial system, and binding of hepcidin to ferroportin causes iron accumulation within the cell resulting in reduced availability of iron for erythropoiesis. The synthesis of hepcidin also leads to inhibition of iron absorption in the small intestine.

Hepcidin may also be involved in the pathogenesis of ‘anemia of inflammation’. Growing evidence suggests that low-grade inflammation causes an increase in hepcidin production, limiting the availability of iron for erythropoiesis and thus providing a direct link between inflammation and metabolism of iron in anemia [33–36]. Malyuszko et al. [37] also reported that serum pro-hepcidin levels and C-reactive protein in HD patients were higher than in healthy volunteers. In another recent study on 50 HD patients (25 nonresponders and 25 responders to rHuEPO therapy) and 25 healthy controls, prohepcidin and soluble transferrin receptor, together with CRP, were good markers of resistance to rHuEPO therapy [38]. On the other hand, Kato et al. [39] measured the peak intensity of serum hepcidin-25, the major form of mature hepcidin, in 24 HD patients by using surface-enhanced laser desorption ionization time of flight mass spectrometry, and compared those between rHuEPO-hyporesponsive and rHuEPO-responsive patients in a cross-sectional study. This group found that serum hepcidin measurement, using currently available assays, was not valid in predicting rHuEPO responsiveness in chronic HD patients [39]. Clearly more work is required to provide a useful therapeutic biomarker and index for treatment monitoring.

Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers

Angiotensin II exerts a direct effect and increases the proliferation of erythroid progenitors in vitro [40]. Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) have been suspected to promote ESA hyporesponsiveness via several mechanisms, including inhibition of angiotensin II-induced EPO release and increased plasma levels of N-acetylserylaspartyl-lysyl-proline that prevents the recruitment of pluripotent hemopoietic stem cells [41, 42]. Qureshi et al. [43] investigated the influence of ACEi/ARB and other anti-hypertensive agents on chronic treatment with rHuEPO in 100 CKD patients. They showed that only ACE inhibitors/ARBs negatively interfere with the effect of ESAs.

Sharples et al. [44] found a significantly higher rHuEPO requirement in the II and ID compared with the DD ACE genotypes in a prospective study including 112 CAPD patients. They suggested that the ACE insertion/deletion polymorphism may determine rHuEPO responsiveness in CAPD patients and should be considered when analyzing the rHuEPO resistance phenomenon. However, in a cross-sectional study including 515 chronic hemodialysis (HD) patients treated with rHuEPO, there was no difference in the use of ACEIs and ARBs between patients with and without EPO resistance [45].

Malnutrition

Interestingly small body size with a low BMI or low cholesterol levels are interrelated with poorer outcomes in maintenance HD patients. The malnutrition-inflammation complex syndrome that refers to the frequently observed association between poor energy intake and inflammatory response has been observed as one of the main causes for the ‘reverse epidemiology’ pattern in chronic dialysis patients [46]. Moreover, an inverse correlation between BMI and anemia control has also been observed [47].

The adipose tissue has a dual effect on erythropoiesis. IL-6 and leptin are both secreted by the adipose tissue and IL-6 is associated with decreased EPO sensitivity while leptin stimulates the human erythroid development in vitro [48].

In contrast, the interaction between fat mass (delimited by dual-energy X-ray absorptiometry) and leptin levels on EPO sensitivity in a post hoc cross-sectional analysis in 166 patients with ESRD. They noted that in the presence of high leptin levels, any inhibitory effects of high truncal fat mass on EPO sensitivity were absent [49]. In an interesting study of 479 African-American HD patients, EPO requirements were reduced and EPO resistance improved in patients with high total adipose tissue and subcutaneous adipose tissue [50].

Hyperparathyroidism

Parathyroid hormone (PTH) potentially influences the bone marrow fibrosis, secretion of erythropoietin by renal peritubular fibroblasts via indirect mechanisms, and sensitivity of erythroid progenitors to erythropoietin. Hilali et al. [17] studied 118 ESRD patients (70.3% HD and 29.7% CAPD) with hyperparathyroidism to assess the response to rHuEPO during anemia treatment and found a strong association between rHuEPO hyporesponsiveness and high PTH levels (patients with iPTH >32 pmol/l were considered to have hyperparathyroid-

ism). Lin et al. [51] noted that hyperparathyroidism in 37 chronic hemodialysis patients was associated with more severe anemia and rHuEPO resistance in a prospective clinical trial. In this study, 16 patients with hyperparathyroidism were treated with calcitriol, while 3 had a surgical intervention. Both calcitriol therapy and parathyroidectomy improved anemia in these ESRD patients with hyperparathyroidism.

Pure Red Cell Aplasia

Pure red cell aplasia (PRCA) is a syndrome characterized by a severe normocytic anemia, reticulocytopenia, and absence of erythroblasts from an otherwise normal bone marrow. This is a very rare cause of ESA resistance in ESRD patients. For example, HD patients who were either hypo- or normoresponsive to epoetin treatment were tested for anti-erythropoietin antibodies. In this study, the prevalence of anti-erythropoietin antibodies in HD patients without symptoms of PRCA was determined by screening sera of 536 patients, using ELISA. Positive results were also verified by radioimmunoprecipitation assay and neutralizing activity was determined by bioassay. Anti-erythropoietin antibodies were detected in 3 hypo- and 3 normoresponsive patients, suggesting that anti-erythropoietin antibodies are not a major cause of ESA hypo- or normoresponsiveness [52].

Similarly, in a multicenter, cohort study of 1,677 patients with incident end-stage renal disease, 57 patients with inadequate EPO response were identified; however, only 2 of these 57 patients were positive for anti-EPO antibodies and one patient had clinical PRCA. In the 1,346 patients without evidence of inadequate EPO response, one patient tested borderline positive for anti-EPO antibodies. The incidence of EPO-induced PRCA and EPO antibodies is found to be very low (1.27/1,000 [95% CI 0.3 to 3.7/1,000]) in this study [53].

Inadequate Dialysis

Dialysis adequacy as measured by Kt/V influences the effect of rHuEpo requirement on anemia management in HD patients [54]. Independent of the use of biocompatible synthetic membranes, rHuEPO was lower in 68 iron-replete HD patients. Thus, adequate dialysis may optimize rHuEPO responsiveness [54].

Interestingly, Movilli et al. [55] also showed that inadequate dialysis was associated with higher epoetin requirements, but increasing Kt/V values above 1.33, had no further effect on epoetin responsiveness in iron-replete HD patients on cellulose membranes and stabilized erythropoietin therapy.

L-Carnitine Deficiency

Another potentially important and challenging factor involved in ESA resistance is L-carnitine deficiency. Carnitine increases the erythroid colony-forming units in mouse bone marrow, suggesting that L-carnitine stimulates erythropoiesis [56]. The palmitic acid ester of L-carnitine (palmitoyl-L-carnitine) stimulates erythropoiesis and it has been shown that L-carnitine deficiency destabilizes erythrocyte membrane and causes a reduction in its survival. Oxidative stress in ESRD patients leads to RBC membrane lipid peroxidation and RBC destruction, thereby worsening anemia [57]. Cytokine-induced alterations in cellular iron homeostasis are suggested to be mediated in part by the increased production of reactive oxygen species (ROS) [58]. This then results in EPO resistance in some HD patients.

Reduced L-carnitine (free carnitine) levels and elevated medium- and long-chain acylcarnitines in HD patients are blamed for rHuEPO hypo- or normoresponsiveness [59]. In a recent cross-sectional study by Reuters et al. [60] on 87 HD patients, including 12 patients with a 'high ERI', a significant negative correlation between L-carnitine levels and ERI was established. Moreover, the ratio of non-acetyl acylcarnitines/total carnitine correlated positively with ERI. These data further support a relationship between carnitine levels and response to rHuEPO treatment, and in particular the importance of the proportion of long-chain acylcarnitines within the plasma carnitine pool.

Malignancy

Occult malignancy and hematologic abnormalities such as myeloproliferative disorders may also account for ESA hypo- or normoresponsiveness in some afflicted patients [61]. ESA hypo- or normoresponsiveness is most commonly observed in myelodysplastic syndromes and least frequently seen in multiple myeloma and chronic lymphocytic leukemia, which are often managed with increased ESA doses [62].

Other Potential Causes

In ESA nonresponder CKD patients, increased numbers of microcytic and anisocytic red blood cells were found compared to responders [63]. In nonresponders, significant changes in membrane protein composition, namely altered ankyrin/band 3 and spectrin/ankyrin ratios were noted [63].

Iron uptake from plasma to erythroid precursor cells is regulated by different proteins: transferrin receptor, hemochromatosis (HFE) protein and DMT1 (NRAMP2/

DCT1). HFE gene mutations are associated with a reduction in the amount of rhEPO necessary to support erythropoiesis in HD patients [64]. Conversely, some DMT1 gene mutations are associated with an inhibition of intestinal iron absorption and a decrease in erythroid cell precursor iron uptake, resulting in hypochromic and microcytic anemia [65]. However, in a study on 63 HD patients, DMT1 gene haplotypes were not associated neither with changes in hematological data and iron status, or in rHuEPO doses required to achieve target Hb levels in CKD patients [66]. Further studies are warranted to assess the association of DMT1 gene mutations/polymorphisms in patients with functional iron deficiency.

Finally, both hyper- and hypothyroidism are thought to be potentially associated with ESA hyporesponsiveness [18, 67].

Management

In an ESA hyporesponsive patient, noncompliance with therapy should be excluded. Then, blood loss should be considered and measuring a reticulocyte count may help in identifying blood loss or hemolysis. In patients with low reticulocyte counts, iron deficiency and inflammation, should be immediately investigated and distinguished with iron studies and serum CRP levels. If iron deficiency and inflammation are excluded, underdialysis, hyperparathyroidism, vitamin B12 and folate deficiencies should be considered. If a patient is receiving ACEi and/or ARB therapy and there is no other clearly identifiable cause for the ESA hyporesponsiveness, cessation of these drugs should be considered unless they are important for underlying cardiovascular disease. Bone marrow examination may ultimately be required in some ESA hyporesponsive patients to exclude bone marrow disorders (fig. 2).

In the absence of an underlying cause, potential therapeutic maneuvers (table 1) such as the ascorbic acid therapy, vitamin E supplementation, statins or oxpentifylline administration, may be tried. The use of high-flux biocompatible membranes may enhance EPO responsiveness [68].

Dialysis Strategies

A study by Ayli et al. [69] examined 48 HD patients who could not reach the target Hb level, despite treatment with at least 200 IU/kg/week subcutaneous rHuEPO [69]. In contrast, the high-flux dialysis group required significantly lower rHuEPO doses to achieve higher Hb levels.

Table 1. Potential treatment options for ESA-resistant anemia

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- Increased dialysis clearance
 - Intravenous ascorbic acid
 - Vitamin E
 - L-Carnitine
 - Statins
 - Oxpentifylline
-

In this study, the beneficial effects of high-flux dialysis were thought to be mediated by enhanced clearance of moderate and high molecular weight toxins. However, in a multicenter, randomized controlled trial of high-flux synthetic versus low-flux cellulose membranes in 84 HD patients with ESA-hyporesponsive anemia, neither Hb levels nor rHuEPO dosages were significantly different between groups [70]. Similarly, Yokoyama et al. [71] analyzed 1,207 subjects from the Japanese arm of DOPPS phase II study and found that Hb levels and erythropoietin doses during the 2-year study period were not affected by dialysis membrane biocompatibility (unmodified cellulose or biocompatible) or flux (standard or high performance).

Molina et al. [72] carried out a prospective study in 107 HD patients to test the hypothesis that ultrapure dialysate can improve the response to darbepoetin and may reduce inflammatory markers. Patients were evaluated for 12 months. At the end of the follow-up period, they determined that use of ultrapure dialysate significantly decreases the ESA resistance index, while Hb levels remained within the established margins with a 34% reduction in the weekly dose of darbepoetin. Reactive protein C levels and endotoxin counts were significantly reduced. As a result, it can be concluded that the bacteriological purity of the dialysate reduces inflammatory markers in patients receiving HD and improves the response to treatment with darbepoetin in renal anemia [72].

Hemodiafiltration (HDF) with on-line endogenous reinfusion is a dialysis technique that employs endogenous reinfusion fluid and performs diffusion, convection and adsorption separately. It eliminates backfiltration and uses an ultrapure dialysate and reinfusate. In a study by Ballabeni et al. [73] that evaluates the clinical and biochemical data of six patients submitted to HDF for >6 months, an increase in hematocrit and reduction in ESA dosage was observed. Increases in both albumin and transferrin levels were also noted. The authors speculated that HDF allows better pro-inflammatory middle mole-

Fig. 2. Management of ESA-resistant anemia in CKD.

cule removal, resulting in improved anemia management [73].

Jirka et al. [74] evaluated HDF data prospectively collected in EuCliD2 from 56 clinics in the Czech Republic, Hungary, Italy, and the UK: 2,564 patients on a thrice-weekly schedule achieving an eKt/VX1.20 were considered in this study. Over 12 months, 394 were treated with HDF and 2,170 with HD. After adjustment for age, gender, co-morbidities, and time on renal replacement therapy, HDF resulted in a significant reduction in mortality risk. However, the potential survival benefit of HDF should be examined in controlled clinical trials.

Ascorbic Acid (Vitamin C)

Parenteral vitamin C improves the release of iron from iron storage deposits, and promotes an enhancement of soluble transferrin receptors and TSAT. Since ascorbic acid acts as a reducing vitamin, it leads to the release of iron from ferritin, and enhances movement of iron to the erythrocytes [75].

In a cross-sectional study of Tarng et al. [76] in HD patients, within 7 days of i.v. supplementation with 2 g of vitamin C, a marked decline in the soluble transferrin receptor concentration, and a simultaneous increase in the TSAT were obtained. Attallah et al. [77] established the effectiveness of parenteral ascorbic acid on Hb, TSAT and CRP levels. Standard therapy combined with 6 months of parenteral ascorbic acid (300 mg with each dialysis session) in EPO-hyporesponsive patients resulted in a significant increase of Hb levels and TSAT. Erythropoietin dose, iron-binding capacity and CRP decreased significantly in the treated but not in the standard therapy control group. Similarly, Shahrbanoo et al. [78] demonstrated that in HD patients with refractory anemia and adequate iron stores, vitamin C improved responsiveness to EPO by expanding iron mobilization and possibly via antioxidative effects in 3 months of study. However, not all studies are positive as Taji et al. [79] did not find a beneficial effect of i.v. ascorbic acid on anemia in a 6-month study. Rather, their patients experienced more adverse events.

While it is claimed that a parenteral dosage of 250–500 mg ascorbic acid following a single dialysis session is harmless, long-term vitamin C supplementation may cause tissue deposition of oxalate and increase in cardiovascular morbidity [80]. Thus, plasma oxalate levels should be monitored on a regular basis.

A recent review suggested that HD patients with poor response to EPO can be initiated on a 2- to 6-month trial of 100 mg i.v. vitamin C administration at the end of the

HD session three times a week [81]. If no response is seen, the dose should be titrated up to a dose of 300 or 500 mg i.v. three times a week for another 2–6 months, with careful monitoring for potential toxicity. Once serum ferritin falls to 300 mg/l, vitamin C therapy should be stopped and i.v. iron administration initiated [81]. Of note, current DOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease does not yet recommend the use of vitamin C (ascorbate) in the management of anemia in CKD patients [11]. No other official position statement or practice recommendations have been produced by guideline-establishing groups [11].

Vitamin E

Oxidative stress is increasingly recognized as a crucial factor for anemia in CKD patients. Vitamin E has reduced CRP and monocyte IL-6 levels in healthy volunteers [82]. It may also reduce oxidative stress induced by i.v. iron administration, and treatment with vitamin E has been shown to reduce dosage requirements for ESA [83]. Moreover, vitamin E increases erythroid colony-forming unit-derived colonies in a dose-dependent manner in a mouse model [84].

Interestingly, Cruz et al. [85] recently examined the effect of vitamin E-coated membranes (VECMs) on anemia in 172 stable chronic HD patients in an open-label multicenter study. After 12 months on VECMs, a significant increase in Hb levels and a decrease in rHuEPO dosage were seen, suggesting that antioxidant properties of VECMs may impact favorably on anemia management in chronic HD patients.

L-Carnitine

In view of the fact that decreased L-carnitine levels may be an etiological factor for ESA hyporesponsiveness, L-carnitine supplementation has been proposed as a potential adjunct to rHuEPO, in the treatment of rHuEPO-resistant anemia. A meta-analysis (in 2002) of 21 clinical trials investigating the effects of L-carnitine treatment on renal anemia has found a significant improvement in response to erythropoietin treatment [86]. Since this meta-analysis, more studies on this controversial topic were published. A double-blind, placebo-controlled study in 29 stable HD patients with secondary L-carnitine deficiency was undertaken to obtain more evidence on the effect of L-carnitine on uremic anemia. For 24 weeks, patients received 20 mg/kg dry body weight of either i.v. L-carnitine or placebo (saline) after each dialysis. The data showed a trend ($p = 0.058$) (compared with placebo) for

improved RBC survival [87]. Kadiroglu et al. [88] evaluated the effect of post dialysis administration of parenteral L-carnitine supplementation on hematological parameters and rHuEPO dose in 34 HD patients. Seventeen patients received rHuEPO therapy alone while another 17 received both rHuEPO therapy and L-carnitine (1 g was injected postdialysis three times a week for 16 weeks). At the end of the study, the total weekly dose of rHuEPO was reduced by 20% ($p < 0.05$) in the L-carnitine group.

The ability of carnitine to reduce oxidative stress via its effects on hemoxygenase-1, which reduces the cytokine-induced alterations in cellular iron homeostasis, may form, at least in part, the basis for a greater sensitivity/enhanced response to ESA treatment in HD patients [89].

However, these are not large clinical studies, and there is still currently insufficient evidence to support the routine use of L-carnitine for any indication in dialysis patients. Although oral L-carnitine has been studied as an ESA adjuvant, there is still need for randomized, controlled clinical trials with the exact role of L-carnitine treating EPO hypo-responsive patients. Since DOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease do not yet recommend the use of L-carnitine for the management of anemia in patients with CKD, in clinical practice, L-carnitine is not yet used routinely [11].

Statins

Since statin therapy decreases CRP levels, they may be considered as potentially effective alternative drugs for treating ESA resistance. Sirken et al. [90] analyzed the effect of statin therapy on EPO requirements in 38 HD patients in a retrospective, single-center study. In the statin group, EPO requirements were decreased by 25%, while Hb, ferritin and albumin levels increased. The effects of treatment of 22 dyslipidemic HD patients with renal anemia with 80 mg fluvastatin for 8 weeks on the circulating serum prohepcidin, and high-sensitive C-reactive protein (hs-CRP) levels were compared with placebo. Total cholesterol, LDL-cholesterol, hs-CRP and serum prohepcidin levels significantly decreased with fluvastatin treatment [91]. A cross-sectional study of Suassuna et al. evaluated the effects of low doses of simvastatin on inflammatory markers, and hematimetric parameters in patients undergoing hemodialysis [92]. In the simvastatin group, there was also a tendency towards reduced resistance to erythropoietin but the difference was not statistically significant [92].

There is clearly a need for randomized control trials to precisely define the utility of statins in ESA resistance.

Oxpentifylline

Oxpentifylline (pentoxifylline) has important anti-inflammatory properties (via inhibition of phosphodiesterase) that are anti-apoptotic, anti-oxidant, anti-TNF- α and anti-IFN- γ actions [93]. Cooper et al. [94] administered oral oxpentifylline (400 mg daily) for 4 months to 16 ESRD patients with EPO-resistant anemia (defined as a hemoglobin level <107 g/l for 6 months before treatment and an rHuEPO dose $\geq 12,000$ IU/week). Among the 12 patients who completed the study, mean Hb concentration increased from 95 ± 9 to 117 ± 10 g/l ($p = 0.0001$). Johnson et al. [95] designed a multicenter study to determine whether oxpentifylline represents a safe and effective strategy for treating erythropoiesis stimulating agent resistance in CKD; however, this study has not yet been completed.

New Therapeutic Options

The role of the protein product of the growth arrest-specific gene 6 (Gas6) for addressing ESA resistance was studied in an animal model. Murine erythroblasts released Gas6 in response to erythropoietin and Gas6 enhanced EPO receptor signaling. Moreover, in the absence of Gas6, erythroid progenitors and erythroblasts were hypo-responsive to the pro-survival activity of erythropoietin [96]. These findings warrant further studies to fully elucidate the therapeutic role of this protein product.

Juzen-taiho-to (TJ-48), a mixture of herbal extracts that is used traditionally to treat patients with anemia or anorexia, has been studied for ESA-resistant anemia treatment. Forty-two HD patients with erythropoietin-resistant anemia were divided into two groups matched for age, sex, serum creatinine, blood urea nitrogen, serum iron, and ferritin. After 12 weeks of 7.5 g/day TJ-48 treatment, a significant increase in Hb level and a significant decrease in CRP were seen in the TJ-48 group. As a result, TJ-48 may be effective in improving erythropoietin-resistant anemia in end-stage renal disease patients [97].

Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) represent potent stimulants of erythroid progenitors, and both hormones stimulate the release of erythropoietin. Therapeutic application of GH and IGF-1 are restricted due to the difficulties of assessing pituitary functional status in CKD patients, the route of administration, its high cost and the interaction with multiple IGF-binding proteins that determine IGF-1 bioavailability.

ity. Zinc supplementation has been found to enhance IGF-1 bioavailability and it may reduce ESA requirements, especially in patients with malnutrition or zinc depletion [98].

Conclusion

Anemia management continues to be a difficult problem in a significant proportion of dialysis patients. Unfortunately, up to 15% of patients have either a blunted or no response to erythropoietin, despite high-dose therapy. Since anemia and rHuEPO resistance contribute to excess morbidity and mortality and generate significant costs, it is important to more completely understand the

etiological factors and treatment modalities available for this entity. Absolute or functional iron deficiency represents the most important cause of ESA hyporesponsiveness followed by infection and numerous inflammatory conditions. Other less-frequent but potentially important factors may also influence responsiveness. Anti-inflammatory and anti-oxidant therapies such as ascorbic acid, vitamin E, statins and oxpentifylline have been studied for the treatment of ESA resistance with variable success. Although promising, all these therapies need confirmation by larger and better designed trials. Improving ESA responsiveness is a relevant objective for minimizing the cost of anemia therapy and improving clinical outcomes.

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