Improving Erythropoiesis Stimulating Agent Hyporesponsiveness in Hemodialysis Patients: The Role of Hepcidin and Hemodiafiltration Online

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Keywords

Erythropoietin stimulating agent hyporesponsiveness · Hepcidin · Erythropoietin · Anemia · Hemodiafiltration online · Chronic kidney disease · Inflammation

Abstract

Hyporesponsiveness to erythropoietin stimulating agents (ESAs) is a condition associated with increased mortality. Even after identifying the condition, the causes are difficult to treat and only partially reversible in end-stage renal disease patients. Thus, the role of more recent hemodialysis (HD) techniques in improving such conditions is an emerging issue. However, major randomized clinical trials have not confirmed the results of smaller observational studies in which online hemodiafiltration has shown some efficacy in improving patients' response to ESAs. In our interpretation, these findings are not in contrast, but they can be explained by a better understanding of the interactions between HD and ESAs on iron mobilization, first of all through the role of hepcidin. The kinetics of hepcidin removal through HD combined with the action of selected ESAs may help the clinician in prescribing the best association between HD treatment and ESAs to overcome hyporesponsiveness.

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Introduction

Erythropoietin-stimulating agents (ESAs) are the milestone in the management of anemia associated with chronic kidney disease (CKD). Since their first use in 1989, ESAs clinical availability resulted in blood transfusion independence and iron overload avoidance in CKD and end-stage renal disease (ESRD) patients [1].

Nevertheless, the individual response to ESAs may vary greatly on the basis of various determinants, and in some cases may be poor or extremely poor, showing significant anemia despite large doses of ESAs. This condition is defined as hyporesponsiveness or resistance to ESAs and is associated with increased mortality [2–4]. Among many factors involved in ESA hyporesponsiveness, we focused on the role of hemodialysis (HD) treatment.

ESA Treatment Options in HD

In HD patients, according to 2012 Kidney Disease: Improving Global Outcomes guidelines, initiation of ESA treatment is suggested for hemoglobin level lower than 10 g/dL with caution for CKD patients with history of stroke or an active malignancy [5], provided that other causes of

Table 1. Current definitions used for hyporesponsiveness to erythropoietin stimulating agents

K/DOQI	450 units/kg per week IV EPO or 300 units/kg per week subcutaneous EPO
European guidelines	300 units/kg per week of EPO (approximately 20,000 units/week) and 1.5 µg/kg per week of darbepoetin alfa (approximately 100 µg/week)
KDIGO	Initial hyporesponsiveness: no increase in hemoglobin concentration after the first month of appropriate weight-based dosing
KDIGO	Acquired hyporesponsiveness: requiring 2 increases in ESA doses up to 50% beyond the dose at which the patient had originally been stable
ERI	Weight-adjusted weekly ESA dose divided by the hemoglobin value

K/DOQI, NKF-DOQI clinical practice guidelines for anemia of chronic renal failure; EPO, erythropoietin; European guidelines; KDIGO, chapter 1: diagnosis and evaluation of anemia in CKD.
ERI, erythropoietin resistance index.

anemia are excluded and iron status has been evaluated and concomitant iron deficiency restored.

Pharmacokinetics and pharmacodynamics of recombinant erythropoietin (rHuEPO) differ on the basis of the type of ESA used and the route of administration chosen. Usually, intravenous administration is the most common among HD patients. rHuEPO isoforms have an half-life of 4–10 h, while second generations ESAs, darbepoetin alfa, and methoxy polyethylene glycol-epoetin beta have a longer half-life (24–26 and 120 h respectively). Thus, the definition of short-acting ESAs for rHuEPO isoforms and long-acting ESAs for the latter. Therefore, in HD patients, rHuEPO are administered up to 3 times per week, while darbepoetin alfa and methoxy polyethylene glycol-epoetin beta weekly to monthly. As a consequence, the bioavailability of short-acting ESAs is characterized by a high and short serum concentration peak immediately after the HD session, while long-acting ESAs ensure a more gradual decrease of serum concentration in a period of weeks.

ESA Hyporesponsiveness

As previously mentioned, an inadequate response to ESAs among CKD patients is an unfavorable prognostic factor. Hyporesponsiveness to ESAs may be present from the beginning of ESA treatment or after the treatment is established. It can be characterized by the requirement of excessive doses during initiation of therapy or by the inability to maintain target hemoglobin levels despite maximum dosage in the iron replete patient. According to different guidelines, ESA hyporesponsiveness is defined as 450 units/kg per week intravenous rHuEPO or 300

units/kg per week subcutaneous rHuEPO (K/DOQI [6]), or 300 units/kg per week of rHuEPO (approximately 20,000 units/week), and 1.5 µg/kg per week of darbepoetin alfa (approximately 100 µg/week; European Guidelines [7]). According to Improving Global Outcomes [5], ESA hyporesponsiveness can be distinguished in initial or acquired. Initial hyporesponsiveness appears to be a failure to increase hemoglobin concentration after the first month of appropriate weight-based dosing. Acquired hyporesponsiveness is defined as requiring 2 increases in ESA doses up to 50% beyond the dose at which the patient had originally been stable (Table 1).

Commonly, patients hyporesponsive to ESAs may also be defined as those patients who receive doses greater than those administered to 75–90% of patients in a given dialysis facility.

In order to normalize the amount of ESAs required depending on the severity of anemia, the determination of Erythropoietin Resistance Index (ERI) may be useful. Currently, ERI is defined as the weekly ESA dose per kilogram of body weight divided by hemoglobin level (gram per deciliter) [8], substituting previously proposed definitions (ESA weekly dose/Hct, ESA weekly dose/body weight/Hct). The predictive value of ERI has been proved to be higher than hemoglobin in many studies [8, 9]. In the Italian study RISCAVID [8], conducted on 700 and 53 HD patients in the north-western area of Tuscany, patients were divided on the basis of basal ERI into quartiles, defining patients belonging to Quartile IV as hyporesponders. In this subgroup, the predictive value of ERI both as continuous and categorical variable for fatal/non-fatal cardiovascular events appeared to be higher than the value of hemoglobin.

Causes of ESA Hyporesponsiveness

The most common cause of resistance to ESAs is insufficient iron availability for erythropoiesis. Absolute iron deficiency may be due to external blood losses and/or exhaustion of iron stores due to an increase in erythropoiesis caused by ESA treatment. Iron-restricted erythropoiesis is a condition determined by unavailability of iron in spite of sufficient iron stores and should be considered in patients who require high doses of erythropoietin, have low transferrin saturation (<25%), and high ferritin [10].

The malnutrition-inflammation complex syndrome or the malnutrition, inflammation, and atherosclerosis syndrome are extremely important determinants of ESA hyporesponsiveness. These conditions arise from a combination of protein-energy malnutrition and a state of chronic inflammation, which alters the metabolic processes and seriously affects the handling of iron [11]. These syndromes may be suspected by concurrent elevation in markers of inflammation and depression in nutritional markers such as albumin and cholesterol and require complex therapeutic approaches to improve. Among the causes to be assessed, periodontal disease, HIV infection, the presence of a failed kidney transplant, an occult infection of an old, nonfunctioning arteriovenous graft [10], or an increased number of vascular access-related infections are associated with higher ESA requirements.

Bone disease due to severe secondary hyperparathyroidism [12], occult malignancy, and unsuspected hematologic disorders [13], as well as hemoglobinopathies such as sickle cell disease and thalassemic trait and the aluminum accumulation in bone, currently rare, may cause ESA resistance. Some reports suggest that the administration of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor antagonists may result in relative ESA resistance due to genetic determinants influencing the effect of these drugs in patients receiving ESAs [14].

Role of HD in ESA Hyporesponsiveness

HD adequacy may affect responsiveness to ESAs. This relationship was suggested by Ifudu et al. [15] in a study of 20 stable patients on maintenance HD who were being treated with rHuEPO and who were modestly under-dialyzed (urea reduction rate <65%). These patients received an increased intensity of dialysis for 6 weeks and showed a rise in hematocrit from 28.4 to 32.3% despite a constant dose of rHuEPO, while the hematocrit did not

change in the control group. In the study conducted by Movilli et al. [16], the same results were obtained independently from the use of biocompatible membranes, confirming that adequacy of dialysis reduces the doses of recombinant erythropoietin. A change in dialysis prescription is not likely to significantly enhance the response to ESAs in patients who are already being adequately dialyzed, as the relationship between dialysis dose and hematocrit is lost at $Kt/V > 1.33$. Use of ultrapure dialysate has been associated with reduced rHuEPO requirements, presumably related to reduction of inflammatory responses [17].

Since the availability of convective HD techniques, some pilot investigations have explored the ESA response comparing standard bicarbonate HD, hemofiltration (HF), and on-line hemodiafiltration (OL-HDF) [18].

High convective volumes combined with high permeability membranes allow an increased clearance of middle-weight molecules, including inflammatory cytokines and peptides possibly involved in ESA resistance.

Role of Heparin in ESA Hyporesponsiveness

In the last decade, the hepatic hormone hepcidin (HEP) has been progressively recognized as the master regulator of the erythroid response to the inflammatory stimulus [19]. Heparin was first identified by Ganz and collaborators in 1998 [20]. They purified a new defensin-like peptide containing 25 amino acids and 4 disulfide bonds and named it hepcidin because its mRNA was highly expressed in the liver and because the peptide showed weak microbicidal activity in vitro. It is encoded by the *HAMP* gene, which produces an 84-amino-acid preprohormone, cleaved to 60-amino-acid prohepcidin, then the mature HEP-25 [21]. Its link to inflammation and innate immunity was noticed from the beginning, as hepcidin concentration was increased more than 100-fold in the urine of a septic donor [20]. The first correlation between iron and hepcidin was described by Pigeon et al. [22], who identified hepcidin mRNA as an iron-induced transcript. Afterwards, ferroportin was identified and demonstrated to be the receptor for hepcidin. Ferroportin is a transmembrane iron channel present in enterocytes, macrophages, and hepatocytes. The binding of hepcidin to ferroportin directly induces the endocytosis of ferroportin and its proteolysis in lysosomes [23]. Thereby, iron transport is hindered, and increased hepcidin levels can cause true iron deficiency by blocking iron release from iron stores. Conversely, low levels of

hepcidin improve iron mobilization from stores, thus raising iron availability to match demand. Heparin is upregulated by both increased iron stores and inflammation, while it is downregulated by iron deficiency, hypoxia, and anemia. Transferrin-bound iron stimulates hepcidin expression in hepatocytes [24]. Inflammatory states with high interleukin (IL)-6 are followed by increased levels of hepcidin and then by hypoferrremia. Hypoxia-inducible factor mediates hypoxia suppression of hepcidin.

In a cross-sectional study of a cohort of the CONTRAST study [25], 25-hepcidin levels were shown to be independently and positively associated with ferritin levels and inversely with residual renal function. Thus, hepcidin represents a common effector of the homeostatic regulation of the intracellular iron fluxes in response to iron stores, erythroid, and inflammatory regulators such as cytokines [26]. 25-Hepcidin is cleared through kidney function, and progression of renal disease has been associated with increased serum hepcidin levels [27].

We believe that, although many factors act differently in determining ESA resistance, a high blood concentration of hepcidin is probably the main final common pathway.

Current Strategies to Improve ESA Hyporesponsiveness

Iron Therapy

Although intravenous iron administration combined with high doses of ESA therapy can overcome some degree of hepcidin-mediated iron blockade, there are some reasons that suggest caution in adopting this strategy routinely. The importance of an adequate iron therapy cannot be underestimated. While iron replenishment leads to a correction of low hemoglobin levels and sparing of ESA, an unguided intravenous iron therapy may lead to iron overload with significant iron deposition and tissue injury.

Current studies have shown high liver-iron content in dialysis patients receiving intravenous iron. The long-term effects of intravenous iron and enhanced iron stores have not been rigorously investigated. Free iron rises after intravenous iron administration, which may promote infections and oxidative damage. In observational studies, the received cumulative intravenous iron dose has been linked to increased risk of death and hospitalization [9]. Similarly, high doses of ESAs should be avoided. Cardiovascular safety findings from several

randomized controlled trials (RCTs) such as CHOIR [28], CREATE [29], and TREAT [30] which targeted Hb values >11 g/dL, drove regulatory authorities to institute warnings to use the lowest ESA dose adequate for reducing the need for red blood cell transfusion. Similar recommendations have also been made based on observational studies.

HD Techniques

The role of HD technique on ESAs resistance treatment is controversial. HD adequacy can improve the inflammatory status and, by lowering hepcidin levels, reduce ESA requirement. In a randomized study of Costa et al. [31] on 50 HD patients, non-responders to ESA treatment had higher C-reactive protein, lower serum albumin levels, as well as a lower number of total and CD4+ lymphocytes, in comparison with responders. This may suggest a correlation between resistance to ESA therapy and the magnitude of the inflammatory response. On the other hand, HD treatment could act removing a significant amount of hepcidin. Lowering hepcidin would release iron from stores, promote erythropoiesis, and prolong red cell survival, and therefore may be safer than high doses of ESAs with repetitive parenteral iron.

Hepcidin is a middle-molecular-weight substance with a molecular weight of 2,791 Da and the removal by dialysis has been demonstrated in adult patients with varying degrees of efficiency [32]. The clearance is mild in low-flux HD, intermediate by high-flux HD, and elevated in OL-HDF. Recently, we demonstrated that the use of new HD techniques and more efficient membranes that allow higher convective volume, allow the removal of larger quantities of hepcidin with a significant reduction in pre-HD levels and a consensual improvement of ESAs resistance [33]. Also Stefansson et al. [34], in a short-term study consisting of a small population of 20 patients, suggested that hepcidin levels were significantly reduced after 60 days of high volume OL-HDF with respect to low-flux HD. The role of membrane permeability in removing high quantity of hepcidin and in reducing its pre-HD levels has been confirmed by Teatini et al. [35] using a high cut-off membrane. In the study group, 50% reduction on hepcidin level was achieved and it was associated with an increase of hemoglobin level. In the control group, the pre-dialysis level of hepcidin did not change. The association of high convective volume and good membrane permeability appears to be the solution to obtain lower levels of hepcidin, better iron release from stores, and more effective erythropoiesis.

Randomized and Observational Studies

Despite these stimulating data, randomized and observational studies designed to investigate the efficacy of OL-HDF in reducing ESA resistance provided conflicting results. We reviewed studies comparing low-flux or high-flux HD versus post-dilution OL-HDF. Among observational studies, Bonforte et al. [36] studied 32 HD patients 3 months before and 3, 6, and 9 months after the transfer to OL-HDF and showed a decrease in ESA consumption with a maximum decrease during the ninth month of follow-up. On the contrary, Oates et al. [37] in an observational study did not find a significant difference between HDF and HD but the design of the study is questionable, the 2 groups were not comparable and the results were not adjusted for any co-variables. Marcelli et al. [38], in a recently published study based on a large population of incident HD and HDF patients matched in order to limit possible bias by indication, compared the dynamics of the erythropoiesis stimulating agent resistance index between HD and HDF. Applying a sensitivity analysis stratifying by type of ESA and administration route, the authors found a clear difference between HD- and HDF-treated patients in ERI reduction limited only to those HDF patients assigned to intravenous rHuEPO treatment and showing a high baseline ERI.

Small sample size randomized studies except Ward et al. [39] showed an improvement of ERI or a reduction in ESAs consumption in post dilution OL-HDF (Lin et al. [40], Vaslaki et al. [41], Schiffl [42], Pedrini et al. [43], and Panichi et al. [33]). The study from Ward et al. [39] failed to find a significant difference between online HDF and high-flux dialysis regarding the ERI but the sample size was very small, and the groups were not well balanced, particularly for the prevalence of polycystic kidney disease (8.3% in the HDF group vs. 23.8% in the HD group). Therefore, most observational studies and small sample size randomized trials demonstrate a positive effect of OL-HDF on ERI consumption.

Randomized Controlled Trials

The results coming from RCTs are more difficult to explain. The CONTRAST study, the Turkish OL-HDF study [44] and the ESHOL study [45] published in the period 2012–2013 are the most important trials designed to compare the effect of OL-HDF over HD on patient survival. Differences on ERI or ESAs consumption were secondary end points for all 3 studies. The CONTRAST study results concerning anemia and ESA resistance have

been published more recently by Van derWeerd et al. [25] ESA was prescribed as epoetin alfa or beta or darbepoetin alfa; 73% of patients at baseline were treated by darbepoetin alfa. ESA and iron supplements were administered via the venous blood line at the end of a dialysis session. During the follow-up period, the mean levels of Hb were 11.9 (11.8–12.0) in OL-HDF vs. 11.7 (11.6–11.8) in HD and ESA index 0.41 (0.38–0.45) in OL-HDF vs. 0.43 (0.39–0.47) in HD. There was a trend toward a lower consumption of ESA in OL-HDF but the value did not reach a statistical significance. The mean dose of iron supplementation during follow-up tended to be slightly higher in the OL-HDF group as compared to HD.

The Turkish OL-HDF study enrolled 782 patients undergoing thrice-weekly HD and randomly assigned them in a 1:1 ratio to either post-dilution OL-HDF or high-flux HD. The majority of the patients receiving erythropoiesis-stimulating agents (92.2%) used a short acting ESA, the remaining ones darbepoetin alfa. During the follow-up period, the ESA dose (U/week) was significantly lower in OL-HDF than in HD and ERI (U/week per kg per g/dL) was reduced by 22% in OL-HDF vs. HD. The designs of CONTRAST study and the Turkish study were similar, as well as the convective volume in OL-HDF groups, while the mean age of enrolled patients (64 vs. 56.5 years) and the percentage of patients receiving ESA treatment at baseline (89 vs. 57%) were different. The study populations had similar baseline levels of C-reactive protein, albumin, and hemoglobin. Ferritin and transferrin saturation levels were adequate in both but higher in Turkish study. In summary, the only strong difference in the 2 studies was the percentage of patients treated by short-acting ESAs (92 vs. 27%).

The ESHOL study adopted a similar design but the patients on OL-HDF reached convective volume much higher than in the previous studies as a result of a higher mean blood flow rate and a longer mean length of dialysis. Differences on ESA consumption and hemoglobin levels were secondary end points. ESA-treated patients were more than 90%. About half of patients were treated with short-acting ESA and the other half with a long-acting agent. No differences in hemoglobin levels, transferrin saturation index, and ferritin levels were reported between the 2 groups during the whole study period. ESA doses too were comparable between the 2 groups.

However, observing online supplementary tables of the Catalan ESHOL study [45], it is possible to differentiate patients treated with short-acting ESAs from those treated with long-acting ESAs. Considered separately, patients treated with short-acting ESAs showed an increase

in ESAs dosage in HD and stability in OL-HDF, achieving a statistically significant difference on ESAs consumption between OL-HDF and HD.

In all these studies, a common denominator to obtain a reduction of ESA resistance on OL-HDF seems to be the use of a short-acting ESAs, as already noticed in observational studies [38].

The Mechanism of ERI Reduction in OL-HDF

OL-HDF and in particular high volume OL-HDF obtains a significant reduction of hepcidin levels. This reduction can be transient or more stable in relation to the efficacy of dialysis treatment, which correlates the magnitude of convective volume and the efficiency of the membrane in removal middle molecules. In the REDERT study, the hepcidin clearance was 103.9 ± 20.3 mL/min in high volume OL-HDF versus 53.3 ± 30.3 mL/min in HD ($p < 0.05$). Significantly lower pre-dialysis hepcidin levels were also observed in high volume OL-HDF treatment after 3 and 6 months with a positive linear correlation between ERI and hepcidin. These data are in contrast with the results of the study by Kuragano et al. [46] who observed a rebound immediately after the end of the session, with hepcidin returning to pre-HD levels after 3 h. Nevertheless, patients were treated by low-flux HD, the post-HD hepcidin levels were evaluated only in 10 patients in a single HD session and the reduction rate was much lower than that in our study.

In agreement with the hypothesis formulated by Marcelli et al. [38], we may speculate that if the reduction of hepcidin levels was strong and stable after dialysis treatment, the advantage associated to OL-HDF would be demonstrated with any type of ESA. Unfortunately, it is possible to achieve a significant reduction in hepcidin blood concentration for a very transient period after the end of dialysis treatment before the hepcidin rebound. Only a short-acting ESA which performs its action very rapidly is able to produce a significant improvement of erythropoiesis under these conditions. Therefore, higher hepcidin clearance combined with a short-acting ESA intravenously administered appears to be the leading mechanism of ESAs resistance lowering on OL-HDF (Fig. 1).

This hypothesis explains the apparently conflicting results of the previously mentioned studies. The improvement in ESA hypo-responsiveness is not to be searched for in a single therapeutic intervention, but rather in the combination of different measures capable of determining and exploiting a low hepcidin status. First, it is fundamen-

Fig. 1. Removal of hepcidin through OL-HDF contributes to iron mobilization. Pre-OL-HDF status is characterized by high levels of hepcidin, which binds to ferroportin and mediates its consequent immobilization of iron intracellular deposits. OL-HDF through high convective volumes and high permeability membranes allows greater removal of hepcidin. Immediately after the OL-HDF session, in absence of hepcidin-binding, ferroportin migrates on the membrane surface allowing iron outflow from cell to blood, available for erythropoiesis.

tal to achieve a significant reduction of hepcidin blood concentration compared to baseline levels. OL-HDF has been demonstrated to obtain a better clearance of hepcidin in patients with high baseline ERI. However, this reduction is not sufficient to improve erythropoiesis. Second, as the reduction of hepcidin blood concentration is of short duration, the erythropoietic stimulus induced by ESAs has to be concentrated in this temporal window. Erythropoiesis induced by subcutaneous ESA is slow and progressive and delayed compared to the time lapse of hepcidin reduction. For the same reason, a long-acting ESA, even if intravenously administered, is inadequate to intercept the favorable low blood hepcidin status, as it acts gradually over a span of weeks. In contrast, intravenous short-acting ESAs at the end of the OL-HDF session seem to be the only agents able to produce their effect in the low hepcidin temporal window and to show efficacy in improving ESA hypo-responsiveness. OL-HDF has therefore an important impact on anemia in ESRD patients, as it gives the basic condition to overcome ESA hypo-responsiveness, given that it is associated with the right ESA

agent and the correct administration route. It is possible to speculate that, in this condition of lower levels of hepcidin and IV ESAs, IV iron administered at the end of the OL-HDF session could be more easily used for erythropoiesis, minimising the risk of toxin accumulation.

An additional mechanism contributing indirectly to lower hepcidin levels is the improvement of inflammatory status by OL-HDF through the reduction of IL-6, a powerful stimulator of hepcidin as previously mentioned. The reduction on IL-6 of is a common finding with OL-HDF techniques. Among these observations, Akoglu et al. [47] demonstrated a significant reduction of IL-6 levels on OL-HDF whereas HD with low- and high-flux polysulfone membranes did not obtain any effect on IL-6 level.

The relevance of hepcidin level reduction in improving erythropoiesis has been recently confirmed by a phase 2, randomized study comparing a new class of drugs, hypoxia-inducible factor-prolyl hydroxylase inhibitors with epoetin alfa. This drug operates by an intermittent activation of genes associated with erythropoiesis and lowering hepcidin level. The study showed a positive impact on iron availability to overcome the therapeutic barrier to erythropoiesis from the inflammatory component present in ESRD [48].

Summary

Analyzing data from major RCTs comparing HD and OL-HDF (CONTRAST study, Turkish study, and ESH-OL study), we can speculate that the observed differences in ESA responsiveness may result from different anemia protocols in terms of ESA type and route of administration rather than from the efficiency of OL-HDF only. Probably, if all RCTs adopted an intravenous short-acting ESA, the results would be comparable.

In HD patients, ESA hyporesponsiveness is a common finding and many factors are involved in determining this condition. The causes should be assessed and treated whenever possible. OL-HDF and in particular high volume OL-HDF coupled with a short-acting ESA is a possible strategy to improve the ESA response in most cases.

In view of the results, OL-HDF may become the first-line option in HD patients with ESA hyporesponsiveness.

Disclosure Statement

The authors have no competing interests.

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