Chapter 16

rhEPO for the Treatment of Erythropoietin Resistant Anemia in Hemodialysis Patients – Risks and Benefits

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Additional information is available at the end of the chapter

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1. Introduction

Anemia is a common complication in hemodialysis (HD) patients, mainly due to the insufficient production of erythropoietin (EPO) by the failing kidneys [1]. Anemia itself can worsens cardiac function, cognitive function, exercise capacity and quality of life, and it has been independently associated with increased mortality and progression of renal disease [2, 3]. A successful management of anemia is, therefore, crucial, as it may improve clinical outcome. The introduction of recombinant human EPO (rhEPO) therapy to treat anemia of chronic kidney disease (CKD) patients reduced anemia, improving patients' quality of life [3]. There is, however, a marked variability in the response to this therapy and 5-10% of patients develop resistance to rhEPO therapy [4]. Resistance to rhEPO therapy has been associated to inflammation, oxidative stress and "functional" iron deficiency, as major causes.

EPO presents also an important protective role in other tissues, outside of the erythropoietic system. Actually, a biological response to EPO and the expression of EPO receptors, have been observed in many different cells, namely, in endothelial, neural and cardiac cells. However, HD patients requiring high rhEPO doses present an increased risk of death [5]. Recently, randomized controlled trials showed no benefit, or even increased risk of mortality and/or cardiovascular complications, in HD patients with hemoglobin (Hb) concentration higher than the target levels [6].

In this book chapter, a review of the etiological mechanisms associated with the development of EPO resistant anemia, in HD patients, will be performed. We also intend to review also the risk-benefits associated with high rhEPO doses used to achieve the target Hb levels.

2. Anemia of chronic kidney disease

CKD is a pathological condition that results from a gradual, permanent loss of kidney function over time, usually, months to years. CKD can result from primary diseases of the kidneys. However, diabetic nephropathy and hypertension have been considered as the main causes of CKD [1]. Anemia is a common complication of CKD that develops early in the course of the disease increasing its frequency with the decline of renal function. The incidence of anemia is less than 2 % in CKD stages 1 and 2, about 5% in CKD stage 3, 44% in CKD stage 4 and more than 70% in the end-stage renal disease (ESRD) [7]. This condition is associated with a decreased quality of life [3], increased hospitalization [2, 8], cardiovascular complications - angina, left ventricular hypertrophy (LVH) and chronic heart failure – and mortality [9-12].

The European Best Practice Guidelines for the management of anemia in patients with CKD recommends that a diagnosis of anemia in these patients should be considered when Hb concentration falls below 11.5 g/dL in women, 13.5 g/dL in adult men and 12.0 g/dL in men older than age 70 [13].

The anemia of these patients is, mainly, due to decreased kidney´s secretion of EPO. In CKD patients there is a failure in increasing the EPO levels in response to hypoxia, as occurs in others types of anemia. These patients present an EPO deficiency, rather than an absolute lack, as EPO remains detectable even in the most advanced stages of CKD [14]. However, other factors contribute to the anemia in these patients, as reduced red blood cell (RBC) life span, iron deficiency, uremic toxins, HD procedure, blood loss and inflammation.

3. Erythropoiesis-stimulating agents

The correction of anemia in CKD patients needs pharmaceutical intervention with erythropoiesis-stimulating agents (ESAs). An intravenous (i.v.) iron supplementation, as adjuvant therapy, should be administrated to prevent iron deficiency and minimize the dose of ESA needed to achieve the target-range of Hb levels [4, 13]. However, recently, some concerns about this treatment of the anemia were raised and questioned in several studies, namely, the need to define Hb targets, safety, benefits and costs of ESA treatments.

3.1. Pharmacology of erythropoiesis- stimulating agents

The introduction of ESAs revolutionized the treatment of anemia in CKD patients. After cloning of the EPO gene, the recombinant human technology allowed the production of ESAs that present the physiological role of EPO. Epoetin beta was the first ESA to be used. It was presented in 1987 [15] and approved by the Food and Drug Administration (FDA) in 1989. Since then, other ESAs appeared, with similar actions, differing in their half-life. Consequently, they were divided in “short-acting” and “long-acting” ESAs (Table 1). The frequency of administration and route of administration (usually, the intravenous (i.v.) administration is more convenient for HD patients) is, therefore, conditioned by their half-life.

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In humans, it seems that the rhEPO treatment increases Hb concentration, and, thus, arterial oxygen content, by increasing red cell volume and depressing plasma volume, probably through a mechanism involving the reduction of the renin–angiotensin–aldosterone axis activity [16].

The mechanisms for ESAs elimination are not well elucidated, and several hypotheses have been considered [17, 18]:

- ESAs are primarily cleared by a hepatic pathway;
- Clearance of ESAs occurs through the kidneys;
- ESAs may be cleared via EPO receptor-mediated endocytosis and subsequent intracellular degradation.

However, other mechanisms, not yet elucidated can be responsible for ESAs elimination.

ESA	Approval		Characteristics	Half-life	Frequency administration
	FDA	EMA			
Short-acting					
Epoetin beta		1989	Identical a.a. and carbohydrate composition to EPO	i.v. 4 - 12 h s.c. 12 – 28 h	3 times/week
Epoetin alpha	1989	1989	Identical a.a. and carbohydrate composition to EPO	i.v. ≈ 5h s.c. ≈ 24h	3 times/week
Epoetin zeta (biosimilar medicine)		2007	Identical a.a. and carbohydrate composition to EPO	i.v. ≈ 5h s.c. ≈ 24h	3 times/week
Epoetin theta (biosimilar medicine)		2009	Identical a.a. and carbohydrate composition to EPO	i.v.≈ 4h s.c. ≈ 34h	3 times/week
Long-acting					
Darbopoetin alpha	2001	2001	2 additional N-linked carbohydrate chains compared to EPO	i.v. 21 hours s.c. 73 hours	once/week
Methoxy polyethylene glycol-epoetin beta	2007	2007	continuous erythropoietin receptor activator	i.v. 134 hours s.c. 139 hours	once/month
Peginesatide	2012		PEGylated, homodimeric peptide with no sequence homology to rhEPO		once/month

Abbreviations: FDA – Food and Drug Administration; EMA – European Medicines Agency; a.a. – amino acid; i.v. – intravenous; s.c. – subcutaneous. rhEPO – recombinant human erythropoietin. Adapted from Food and Drug Administration (2012) [19], European Medecines Agency (2012) [20] and Green et al. (2012) [21].

Table 1. Erythropoiesis – stimulating agents.

3.2. Non-hematopoietic actions of erythropoietin and erythropoiesis- stimulating agents

ESAs are designed to treat anemia, but recent evidences points to other non-hematopoietic actions of EPO and ESAs [22]. Several pleiotropic effects have been attributed to EPO, such as cytoprotective, antiapoptotic, anti-inflammatory and angiogenic capacities.

The erythropoietic and non-erythropoietic effects of EPO appear to result from the existence of two different receptors with different affinities for EPO [23].

In erythroid cells, picomolar concentrations of EPO bind to the EPOR homodimers, whereas on other cells and tissues EPO binds to an heterodimer receptor, constituted by EPOR and CD131 (beta common receptor – βcR), and, high local EPO concentrations are needed to exert its action [23-25]. The EPO variants, including asialo-EPO, carbamylated EPO (CEPO) or carbamylated darbopoetin alpha (C-darbe), that present the protective effects of EPO in non-haematopoietic tissues, but no hematopoietic activity [26-28], suggested the presence of two types of receptors. EPOR are present in several cells and tissues, as brain (neurons, astrocytes, and microglia) [29, 30], kidney [31], female reproductive system [32], vascular endothelial cells [33], cardiomyocytes [34], lymphocytes and monocytes [35], among others.

Some of the non-hematopoietic effects of EPO are summarized:

- Cardioprotection: several studies showed that ESAs promote cardioprotection through the inhibition of cardiomyocyte apoptosis, reduction of inflammation and oxidative stress, and induction of angiogenesis [22-24, 34, 36].
- Anti-inflammatory properties: EPO and its derivates reduce the production of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, and NO (nitric oxide) via inducible NO synthase (iNOS) through the inhibition of NF-κB pathway [23, 24, 37].
- Neuroprotection: EPO seems to be important for the neural development, as it stimulates the differentiation of neural progenitor cells [29], but it also promotes angiogenesis and reduces inflammation, oxidative stress and neuronal apoptosis in some conditions, as hypoxia-ischemia (HI), stroke and neurotoxicity of glutamate [22-24, 29].
- Angiogenesis: EPO increases the number of functionally active endothelial progenitor cells (EPCs), enhancing angiogenesis, and seems to be dependent on functional endothelial NO synthase (eNOS) [24, 38]. EPO plays an important role in uterine angiogenesis, through EPOR expressed by endometrial vascular endothelial cells [33].
- Immunomodulation: EPO may have effects on dendritic cells [potent antigen presenting cells (APCs) that possess the ability to stimulate naïve T cells], presenting effects in innate immunity [39].
- Renoprotection: several studies on acute kidney injury reported that a single dose of rHuEPO reduces kidney dysfunction through an antiapoptotic mechanism, and increased NO production, but only in intact vessels [31]. However, it appears that this renoprotection is achieved only with low doses of EPO, non-hematopoietic doses, as high EPO doses cause an increase in hematocrit that is accompanied with changes in hemorheology, activation of thrombocytes and increased platelet adhesion to injured endothelium [31].

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3.3. Benefits of erythropoiesis-stimulating agents

ESAs have beneficial effects by correcting anemia and their associated symptons (fatigue, dizziness, shortness of breath, among others), improving the quality of life of these patients [40-42]. ESAs also reduce the need for transfusions, thereby reducing transfusion reactions (immunological sensitization), transmission of infectious agents and iron overload [43].

The anemia of CKD is associated with cardiovascular complications, due to increasing blood pressure and LVH. Indeed, LVH is present in many patients with CKD, even in the earlier stages of the disease (75% of patients who start HD have LVH) and may lead to heart failure, cardiac arrhythmia or both, that are considered as major causes of cardiac-related deaths in this population [44, 45]. LVH is a physiological adaption that results from long-term increase of myocardial work, from high-pressure or volume overload, which can lead to major cardiac events. Volume overload can result from anemia, as hypoxia and the decreased blood viscosity contribute to decrease peripheral resistance, and from increased venous return, both of which increase cardiac output [44, 46]. LVH is also a risk factor for the development of uremic cardiomyopathy, which is defined as congestive heart failure due to a primary disorder of the heart muscle in uremic patients, and is characterized by profound systolic dysfunction and cardiac fibrosis; however, increased sympathetic activity in response to anemia also appears to be a factor for this condition [47, 48].

Several studies report the synergy between anemia and LVH and that the use of ESAs for anemia correction (Hb target of approximately 11 g/dL) is associated with an improvement in heart failure symptoms and with a reduction in LVH [45, 49].

The effects of ESAs on the progression of renal function are controversial. Some studies demonstrated that following ESA initiation renal function declines at a slower rate and delays the dialysis initiation in pre-dialysis patients [50-52], while other studies reported that ESAs do not significantly slow renal function decline [53, 54].

3.4. Risks associated with erythropoiesis-stimulating agents

As referred, ESAs have several benefits beyond the treatment of anemia; however, its administration seems to associate some risks. Cardiovascular and thromboembolic events have been described. Some of the protective effects of EPO and ESAs, as described above, occurs upon the activation of the heterodimeric EPOR; however, as the affinity of EPO for this receptor is low, higher doses of EPO are needed to reach these effects.

One of the most described effects of ESAs is hypertension. Several mechanisms can explain the rise in blood pressure (BP) mediated by ESAs. Renal anemia is a factor predisposing to increase BP, due to the increased sympathetic activity and impaired NO availability [55]. ESAs impair the balance between vasodilating and vasoconstrictor factors, since it induces the production of vasoconstrictors as endothelin-1 (ET-1), thromboxane (TXB2) and prostaglandin 2α (PGF2α), and reduces the production of the vasodilatory prostacyclin (PGI2) [56, 57]. Chronic treatment with ESAs appears to impair the vasodilatory capacity of endothelial NO, through an increase in the asymmetrical dimethylarginine (ADMA), an inhibitor of eNOS [57]. ESAs seem to induce hypersensitivity to angiotensin II, a recognized vasoconstrictor [56, 57].

An increase in noradrenaline concentration and hypersensitivity - a vasoactive substance - may contribute also to hypertension during ESA therapy [56, 57].

Treatment with ESAs is associated with an increase in the incidence of thrombotic events [58]. EPO has the capacity of stimulating thrombopoiesis, increasing platelet count; however, EPO also increases platelet reactivity (especially on the newly synthesized ones) promoting a prothrombic effect [59]. Some other hemostatic disturbances have been described, as an increased expression in E selectin, P selectin, von Willebrand factor and plasminogen activator inhibitor-1, which may favor bleeding episodes, and increase the risk of thrombosis and thromboembolism, as occlusion of the vascular access [57].

An uncommon but serious complication associated with ESAs administration is pure red blood cell aplasia, an immunogenic side effect that results from the production of antiEPO antibodies induced by ESAs administration [60-62]. Indeed, the method used to produce ESAs may not eliminate impurities or aggregated protein that may trigger the immune response in patients [62]. Immunoprecipitation assays have shown that antiEPO antibodies are directed against the protein moiety of the molecule [61].

ESAs are also indicated in the treatment of symptomatic anemia in adult cancer patients with non-myeloid malignancies receiving chemotherapy. However, some evidences point that these agents can accelerate tumor growth, but data are controversial. High doses of EPO can stimulate endothelial and vascular smooth muscle cell proliferation and promote angiogenesis. The antiapoptotic pleiotropic effect of EPO can also contribute to tumor progression [57, 63].

4. Resistance to erythropoieses-stimulating agents

Although the majority of CKD patients respond adequately to ESAs, 10% of these patients develops resistance to this therapy [4]. According to the European best practice guidelines for the management of anemia in patients with chronic renal failure [13] resistance to ESAs is defined as a failure to achieve target Hb levels (11– 12 g/dl) with doses lower than 300 IU/kg/ week of epoetin or 1.5 μg/kg/ week of darbopoietin-α. For the National Kidney Foundation Disease Outcomes Quality Initiative (NKF KDOQI) guidelines [4], hyporesponsiveness to ESAs therapy is defined by, at least, one of these situations: a significant increase in the ESA dose required to maintain a certain Hb level, a significant decrease in Hb level at a constant ESA dose or a failure to increase the Hb level to higher values than 11 g/dL, despite the administration of an ESA dose equivalent to epoetin higher than 500 IU/kg/week.

ESAs resistance is associated with poor outcome, increasing the risk of mortality [5, 64, 65]. Hyporesponsiveness to ESAs therapy can have many underlying causes. The most common causes are iron deficiency (absolute or functional), and inflammation.

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4.1. Iron deficiency

Iron-restricted erythropoiesis is frequent in CKD patients and is due to absolute or functional iron deficiency. The latter seems to be the most common cause of hyporesponsiveness to ESAs in HD patients [66, 67]. About 25-37% of CKD patients with anemia present with iron deficiency [66]. Iron therapy is recommended, and i.v. iron supplementation is more effective than oral supplementation in HD patients [67]. It is important to distinguish between absolute and functional iron deficiency. Indeed, there is a controversy about iron supplementation when transferring saturation is lower than 20% and ferritin is higher than 500ng/mL (functional deficiency) [67, 68]. In this situation, probably associated with an inflammatory response, an excess of iron can be potentially harmful to these patients.

4.2. Chronic blood loss

Blood loss is frequent in patients undergoing HD and could be a cause to an inadequate ESA response. This condition should always be suspected in several conditions, namely, in patients who need a higher dose of ESA to maintain a stable Hb concentration, in patients whose Hb concentration is falling, and in patients who fail to increase iron stores, even after i.v. iron supplementation [13].

4.3. Inflammation

The anemia of CKD is often referred as an inflammatory anemia. Indeed, inflammation is a common feature in CKD patients, mainly, in those under HD. Inflammation is recognized as one cause to hyporesponsiveness to ESA therapy, and several studies reported an association between high levels of inflammatory markers and ESA resistance in CKD patients [5, 69-72]. Usually, HD patients present with high levels of inflammatory markers, namely, IL-6, CRP, TNF-α, INF-γ, and with lower serum levels of albumin [69-71].

A week response to ESA also appears to be associated with enhanced T cell capacity to express IFN-γ, TNF-α, IL-10, and IL-13 [70, 73]. Costa et al. [71] also reported a significant rise in neutrophil count in non-responder patients. They also found positive correlations between CRP and elastase and between elastase and rhEPO doses, suggesting that elastase, a neutrophil protease released by degranulation, could be a good marker of resistance to rhEPO therapy in HD patients under hemodialysis. Inflammation contributes to anemia through several ways:

- suppression of erythropoiesis: **directly**, by the inhibitory effects of pro-inflammatory cytokines: IL-1β and TNF-α stimulate the growth of early progenitors BFU-E, but suppresses the growth of the later stages, inducing apoptosis in CFU-E [74]; **indirectly** as IL-1β and TNF-α stimulate the production of INF-γ [75], known to mediate erythropoiesis suppression.
- accelerated destruction of erythrocytes (as referred above in the uremic toxins section) by the reticulo-endothelial macrophages activated by the inflammatory state [76];
- reduction of EPO production: in hypoxic conditions, IL-1β and TNF-α increase the expression GATA and NF-κB, both inhibitory of the transcriptional factors of EPO gene [77];

- impaired iron availability for erythropoiesis: transferrin receptors in erythroid and non erythroid cells can be down-regulated by inflammatory cytokines reducing iron uptake [76]; they can also increase the expression of lactoferrin receptors and reduce the expression of ferroportin in macrophages, increasing the iron storage in these cells and reducing the iron availability [76, 78]; inflammation is responsible for the increase of hepcidin expression, a regulatory peptide in the iron cycling that reduces iron absorption and mobilization.

Recently, it was reported the existence of a soluble form of the EPOR (sEPOR) [79, 80]. Although this soluble receptor is able to bind to EPO, the role of these circulating sEPOR in humans remains largely unknown. sEPOR seems to be increased in patients receiving high ESA doses [79, 80], and the pro-inflammatory cytokines IL-6 and TNF-α can be responsible for this increment [79]. sEPOR could, therefore, be associated with ESA resistance through the inhibition of EPO effectiveness.

4.4. Decreased hepcidin excretion

In the last years hepcidin emerged as a key regulator of iron metabolism. Hepcidin is a peptide (25 aminoacids) produced, mainly, in hepatocytes, although other sites of production have been described, such as kidney [81], adipose tissue [82], brain [83] and heart [84, 85]. Hepcidin expression is regulated by the *HAMP* gene located in the long arm of chromosome 19 [86].

An increase of hepcidin levels leads to a decrease in iron absorption (hepcidin inhibits DMT1 transcription [87] or promotes an ubiquitin-dependent proteasome degradation of DMT1 [88]) and an inhibition of iron release from its storages (macrophages and hepatocytes) as hepcidin binds to ferroportin (the only known iron exporter in the cells) promoting its internalization and degradation in lysosomes (Fig. 1) [89, 90].

Hepcidin is increased in HD patients [91, 92], and it is regulated by inflammation [93] and linked to ESA resistance. Hepcidin correlates with IL-6, the cytokine that stimulate its production [94, 95], and with ferritin reflecting high inflammation and high levels of iron stores [96]. Some authors point that hepcidin could be a marker of functional iron deficiency [86] and that ESA therapy can decrease hepcidin levels [72, 96].

The kidney appears to play a role in the excretion of hepcidin, as this peptide is found in urine [97]. Hepcidin levels are increased in HD patients, and its levels appears to be reduced after HD procedure, supporting the role of kidneys in the excretion of this peptide [91, 92].

4.5. Secondary hyperparathyroidism

The parathyroid hormone (PTH) is considered by EUTox Work Group [98] as a middle molecule uremic toxin with some biological effects. Secondary hyperparathyroidism is a condition resulting from the deregulation of calcium and phosphorus homeostasis in the kidney. It seems that PTH could be a marker of hyporesponsiveness to ESAs in dialysis patients [99, 100].

Several mechanisms have been proposed as interference with RBC production as PTH causes bone marrow fibrosis, has an inhibitory effect on BFU-E and interferes with EPO endogenous

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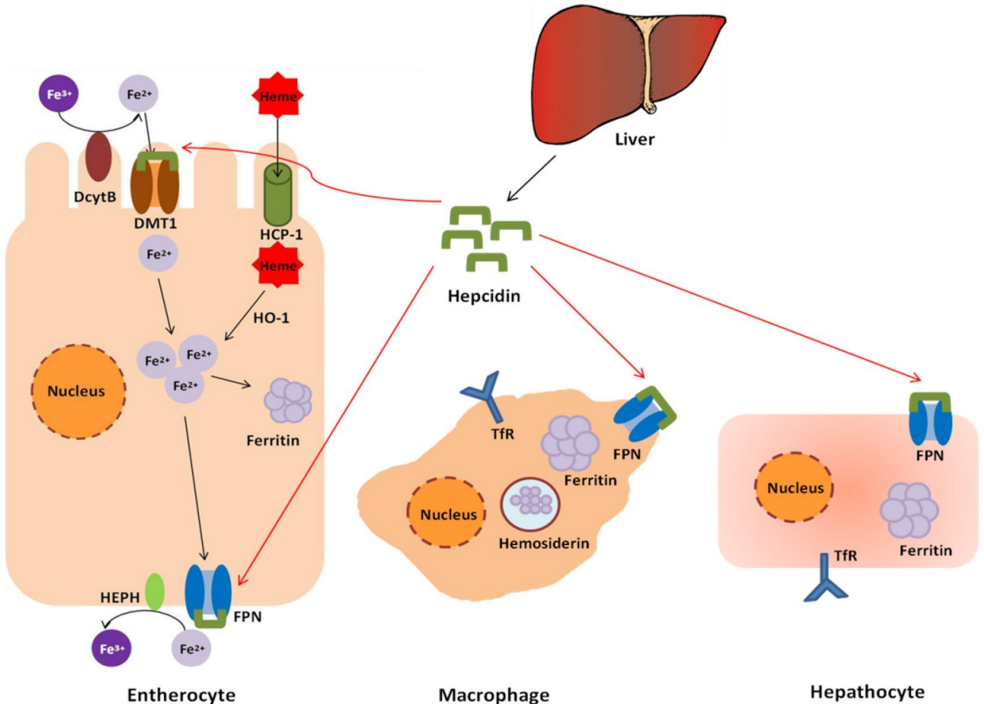

Figure 1. Iron metabolism and hepcidin. The iron is present in the diet as either heme iron (Fe^{2+}) or nonheme iron (Fe^{3+}). Nonheme iron must first be reduced to Fe^{2+}, by duodenal cytochrome B (DcytB), before it can be transported by the divalent metal iron transporter 1 (DMT1). Once inside the enterocyte, the newly absorbed iron enters the intracellular iron pool. If the iron is not required by the body, it is loaded onto the iron storage protein ferritin. Iron required by the body is transferred across the basolateral membrane by ferroportin (FPN). The export of iron also requires the ferroxidase hephaestin (HEPH). Heme carrier protein (HCP1) can transport heme; the enzyme heme-oxigenase 1 (HO-1) is required for releasing iron from heme. Hepcidin expression in the liver inhibits iron absorption from the diet and the release of iron from its storage.

production [99, 101-103]; interference with RBC survival as PTH increases osmotic fragility of erythrocytes [102, 103].

4.6. Aluminium toxicity

Although the recent progresses in the dialysis procedures, some patients present high levels of aluminium (Al) [104]. Usually, high levels of Al cause a microcytic, hypochromic or normochromic anemia that is hyporesponsive to ESA therapy, as it interferes with the enzymes necessary for the heme synthesis [67, 105]. The sources for the increase in plasma Al levels seems to be the water used for dialysis [105], medications given i.v. [104] and infections [106].

4.7. Vitamin deficiencies (e.g. folate or vitamin B12 deficiency)

The deficiency of folate or vitamine B12 is not very common in dialysis patients, but as these nutrients are water soluble and can be easily loss during dialysis, they can become a cause of ESA resistance, especially in patients with malnutrition. The supplementation of these nutrients seems to overcome ESA hyporesponsiveness [66, 67].

4.8. Malnutrition

Low body mass index (BMI) and low levels of cholesterol are related to poor outcomes in dialysis patients, increasing the risk of mortality [107]. This phenomenon, called as "reverse epidemiology", is based on the malnutrition-inflammatory complex [108]. These patients present a decreased nutritional reserve, reducing its capacity to overcome inflammation; they also present a reduced protein-calorie intake, chronic acidosis and failure of vascular access [108]. A diminished nutritional status and the enhancement of inflammation could be responsible for the requirement of higher EPO doses [69, 108].

4.9. Inadequate dialysis

Intensity or adequacy of dialysis (measured by Kt/V) is a factor that can modulate the response to ESA therapy. Inadequate dialysis is associated with the need for higher ESA doses. Some studies showed that convective treatments present benefits in ESA response, as compared with other treatments [109]. High flux HD (HF-HD) and online hemodialfiltration (OL-HDF) improve the response to ESAs, as compared to low flux HD (LF-HD), probably due to a better removal of middle and large molecules that impair erythropoiesis [67, 92, 109]. However, some studies failed to reach to these conclusions [110].

4.10. Angiotensin-converting enzyme inhibitors and angiotensin receptors blockers

These drugs, used for hypertension control, can be associated with ESA hyporesponsiveness due to its effects on angiotensin II. They can act through several mechanisms, not well understood, including inhibition of angiotensin-induced EPO release and increased plasma levels of N-acetyl-serylaspartyl-lysil-proline that impairs the recruitment of pluripotent hemopoietic stem cells [66, 67].

4.11. Testosterone deficiency

It appears that low testosterone levels may contribute to anemia in men with CKD and to ESA resistance. Testosterone stimulates erythropoiesis through the production of hematopoietic growth factors and possible improvement of iron bioavailability [111, 112].

5. Controversies in the treatment of anemia in chronic kidney disease

Since the introduction of ESAs therapy a demand exists to define the better Hb target associated with lower CV risks. Indeed, recent studies reported increased CV risk and death in patients

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treated with high doses of EPO to achieve higher Hb levels, and this led to the controversy of what is the cause of these increased risk: higher doses of EPO or higher levels of Hb?

5.1. Clinical trials

The correction of anemia to higher target Hb levels with ESAs in CKD or ESRD patients merits attention, as it may be associated with increased risk of death or of CV events, namely, stroke, hypertension, and vascular access thrombosis [6].

Only four studies assessed properly the effect of higher Hb levels on the increased risk of CV events and/or death.

5.1.1. Normal hematocrit trial (NHT) [113]

This study included patients under HD with congestive heart failure or ischemic heart disease. They were randomized to one of two groups to receive epoetin alpha, aiming to achieve and maintain a target hematocrit (Ht) of 42% or 30%. Primary end points were the length of time to death or for the first nonfatal myocardial infarction (MI). The study was interrupted due to the increased number of deaths observed in the high-Ht group and that were nearing the boundary of statistical significance. An increased rate of incidence of vascular access thrombosis was also reported in the high-Ht group. The study failed to reach statistical difference between the two groups, however, it was concluded that a target Ht of 42% is not recommended in HD patients.

5.1.2. Cardiovascular risk reduction by early anemia treatment with epoetin beta (CREATE) [53]

This study included pre-dialysis patients in stage 3 or 4 with mild-to-moderate anemia. They were randomly assigned to normalization of Hb values (13.0-15.0g/dL) or to a partial correction of anemia (10.5-11.5 g/dL), in order to investigate the effect of Hb correction on complications from CV causes. The primary endpoint was the time for the first CV event. Secondary objectives included the investigation of the effects of these treatments on the left ventricular mass index, the progression of CKD, and the quality of life. They did not find a significant difference in the risk for a first CV event between the two groups. However, this study reported a higher incidence of hypertension and headaches, and a higher risk for starting dialysis in the group aiming normalization of Hb values. But they also reported significant benefits on the quality of life for the patients with higher Hb targets.

In conclusion, they found that in pre-dialysis patients with mild-to-moderate anemia, the normalization of Hb levels to 13.0 to 15.0 g/dL did not reduce CV events.

5.1.3. Correction of hemoglobin and outcomes in renal insufficiency (CHOIR) [114]

Non-dialysis patients with CKD were included and the effect of raising Hb concentration with epoetin alpha to a target Hb value of 13.5 g/dL or 11.3 g/dL was compared. The primary end point was the time of death, MI, hospitalization for congestive heart failure (excluding renal replacement therapy), or stroke.

An increased risk of the primary end point, for the high-Hb group, as compared with the low-Hb group was found. Death and hospitalization for congestive heart failure accounted for 74.8% of the events. An increased rate of thrombotic events was also reported in the group of high-Hb. Patients in the high-Hb group had a higher (but not significant) rate of both progression to renal replacement therapy and hospitalization for renal replacement therapy. They did not find any apparent additional benefit in quality of life. In conclusion, they recommended the use of a target Hb level of 11.0 to 12.0 g/dL rather than a level of 11.0 to 13.0 g/dL, because of the increased risk, increased costs, and no quality-of-life benefit.

5.1.4. Trial to reduce cardiovascular events with aranesp therapy (TREAT) [115]

In this trial patients with type 2 diabetes mellitus, CKD and anemia were enrolled. Patients were randomized to receive darbepoetin-alfa (in order to achieve a target Hb of 13.0 g/dL) or placebo (in this group were prescribed blinded "rescue" darbepoetin for Hb level < 9.0 g/dL). The primary end point was time to death or hospitalization for myocardial ischemia. A significantly higher rate of strokes in patients treated with darbepoetin was observed. A higher rate of both thromboembolism and cancer-related deaths among patients with a history of cancer in the treatment group was also reported in the treatment group.

Higher targets of Hb levels imply the use of higher ESA doses. Therefore, the increased risk for adverse CV outcomes could also result from the higher ESAs doses and not only from the normalization of Hb [116]. In this sense, a trial has been designed to identify the potential benefits and harms of different fixed doses of ESA. The Clinical Evaluation of the DOSe of Erythropoietins (C.E. DOSE) trial [117] enrolled HD patients that were randomized 1:1 to 4000 IU/week *versus* 18000 IU/week of i.v. epoietin alfa or beta, or of any other ESA in equivalent doses. The primary outcome was death, non fatal stroke, non fatal MI and hospitalization for CV causes.

Several potential mechanisms for harm with higher Hb targets have been proposed and revised by Fishbane et al. [118]. The hypothesis is that increased viscosity and hemoconcentration, the increased BP, the toxic effect of iron and unphysiological doses of ESAs contribute to ESAs toxicity. The rise in Ht results in a higher viscosity and, consequently, higher risk of thomboembolism. It also favors platelet activation by increasing the interaction between the endothelial cells and platelets in blood vessels. Hemoconcentration is a phenomenon observed in these patients after a dialysis session that results from the removal of large amounts of fluids.

5.2. Safety advisories

Considering the results of these studies, in 2007 the FDA launched a safety advisory, recommending that patients do not exceed the Hb level of 12g/dL [119]. At the same time, the NKF KDOQI made an update on its guidelines, recommending that the selected Hb target should generally be in the range of 11.0 to 12.0 g/dL, but should not be greater than 13.0 g/dL [120].

In 2010, the European Best Practice Guidelines Work Group published the recommendation that "Hb values of 11-12 g/dL should be generally sought in the CKD population without intentionally exceeding 13 g/dL" [121]. In 2011, the FDA introduced warnings in the ESA label

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giving the recommendations "for more conservative dosing of Erythropoiesis-Stimulating Agents (ESAs) in patients with chronic kidney disease (CKD) to improve the safe use of these drugs" [122].

5.3. Hemoglobin variability

In conjugation with the optimal Hb target and ESA dose, there is a study of Hb variability (Hb-var). It was noted that during the treatment of HD patients with ESAs the level of Hb have a great fluctuation, that is, the Hb levels tends to rise or fall in a cyclic pattern, that is different for each patient [123]. However, the impact of this Hb-var is not still elucidated. Some studies show that there is an association between Hb-var and increase of death [11, 64, 65], especially if this variability is greater than 1g/dL [11]. The main factor for this variability is ESA dose; however, other factors have been pointed, as i.v. iron and other biological factors (inflammation and nutritional status) [123].

Hb-var represents an important physiological stress, as the ESA treatment involves short, intermittent burst of plasma EPO availability that do not coincide, either temporally or in magnitude with its physiological action. Under physiological conditions EPO levels are maintained in a narrow range, through several mechanisms, in order to support a constant oxygen supply to the organs. The impact of Hb-var on the organism is not fully understood, but the myocardium may be one of the most affected organs, as it has to compensate with an increased output and cardiomyocytes proliferation during the periods of reduced oxygen availability, that occur when Hb reaches lower levels, before the new ESA administration. This might result in deregulation of cardiac growth signal, leading to left ventricular dilation and hypertrophy [11, 123]. The autonomic nervous system can also suffer from this Hb-var; actually, autonomic dysfunction occurs in other pathological conditions, where Hb-var also occurs, like sickle cell anemia [11]. Fishane et al. also [123] found that better responders to ESA tend to have a higher degree of Hb-var.

6. Conclusion

Despite all the technologic advances in HD procedure and medical support, the morbidity and mortality in CKD patients remains high, particularly in hyporesponsiveness patients to ESAs therapy. The clinical trials showed that a higher Hb target is associated with increased risk of cardiovascular complications and death; however, the impact of higher ESAs doses to achieve higher Hb targets remains unclear. Some evidence points that the pleiotropic effects of ESAs can contribute to the ESAs toxicity observed with higher doses. Meanwhile, the recommendations to target Hb to a range of 11 – 12 g/dL, without exceeding the 13g/dL, with the lower doses of ESAs to accomplish this goal, can reduce the risks associated with higher Hb target and higher ESAs doses in CKD patients. More studies are needed on this field to evaluate the impact of the linkage anemia/high sustained ESAs therapeutic doses in CKD that might explain the high mortality in hyporesponsiveness patients. To accomplish these goals blood, cellular and tissue studies are need that cannot be performed in humans; therefore, the use of appro-

priate animal models could be useful to understand whether the association of moderate anemia and high sustained therapeutic doses of ESAs in non-responders is beneficial or an increasing risk; to clarify the underlying mechanisms and, eventually, to propose new therapeutic strategies to reduce mortality in HD patients.

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