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Update article

Resistance of dialyzed patients to erythropoietin



Michelle Teodoro Alves^a, Sandra Simone Vilaça^b, Maria das Graças Carvalho^a,
Ana Paula Fernandes^a, Luci Maria Sant'Ana Dusse^a, Karina Braga Gomes^{a,*}

^a Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil

^b Hospital Felício Rocho, Belo Horizonte, MG, Brazil

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ABSTRACT

Resistance to recombinant human erythropoietin is a common condition in dialyzed patients with chronic kidney disease and is associated with more hospitalizations, increased mortality and frequent blood transfusions. The main cause of hyporesponsiveness to recombinant human erythropoietin in these patients is iron deficiency. However, a high proportion of patients does not respond to treatment, even to the use of intravenous iron, which indicates the presence of other important causes of resistance. In addition to the iron deficiency, the most common causes of resistance include inflammation, infection, malnutrition, inadequate dialysis, and hyperparathyroidism, although other factors may be associated. In the presence of adequate iron stores, other causes should be investigated and treated appropriately.

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Introduction

Chronic kidney disease (CKD) is considered a public health problem worldwide with high incidence and prevalence rates.¹ In end-stage renal disease (ESRD), renal function must be replaced by dialysis or renal transplantation.² In Brazil, the number of patients on dialysis has increased gradually over the years. According to the Sociedade Brasileira de Nefrologia (SBN), 42,695 and 100,397 patients were under dialysis in 2000 and 2013, respectively.³

Anemia is one of the most frequent early complications of CKD.⁴ The main cause is erythropoietin (EPO) deficiency due to impaired kidney function. However, other causes

should be considered when the severity of anemia is inconsistent with the decrease in renal function; when there is evidence of iron deficiency or matching decreases in hemoglobin, leukopenia and/or thrombocytopenia are also found.⁵

The treatment of anemia in CKD patients usually involves the use of recombinant human erythropoietin (rHuEPO). The main cause of rHuEPO treatment failure is the loss or low iron availability.⁶ The prevalence of iron deficiency is very common in CKD, affecting as many as 50% of patients.⁷ However, despite rHuEPO and intravenous iron in the majority of patients, the prevalence of anemia reaches 34% in Brazil.⁸ This indicates the existence of other important factors related to rHuEPO resistance.

* Corresponding author at: Av. Antônio Carlos, 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil.

E-mail address: karinabgb@gmail.com (K.B. Gomes).

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Table 1 – Definition of anemia and hemoglobin target in CKD patients.

	EBPG 2004	NKF/KDOQI 2006/2007	ERBP: anemia group position 2008	KDIGO 2012
<i>Definition of anemia</i>				
Females	<11.5 g/dL	<12 g/dL	<12 g/dL	<12.0 g/dL
Males	<13.5 g/dL	<13.5 g/dL	<13.5 g/dL	<13.0 g/dL
	<12 g/dL aged >70 years			
<i>Hemoglobin target</i>	>11 g/dL >14 g/dL not desirable (>12 g/dL not desirable in CVD)	Generally 11–12 g/dL, not to exceed 13 g/dL	Generally 11–12 g/dL, not to exceed 13 g/dL	Generally ≤11.5 g/dL, not to exceed 13 g/dL

EBPG: European Best Practice Guidelines; NKF/KDOQI: National Kidney Foundation Kidney Disease Outcomes Quality Initiative; ERBP: European Renal Best Practice; KDIGO: Improving Global Outcome; CVD: cardiovascular disease.

The definition of anemia in CKD patients has changed with some guidelines being produced over the last few years. In 2004 the Revised European Best Practice Guidelines (EBPG) on Anemia defined low hemoglobin levels as values <11.5 g/dL in adult females and <13.5 g/dL in adult males (<12 g/dL in over 70-year olds). Patients with CKD should maintain a hemoglobin level >11 g/dL (hematocrit >33%). In addition, levels >12 g/dL are not recommended for patients with severe cardiovascular disease.⁹

An update of the 2006 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF/KDOQI) guidelines in 2007 suggested that anemia is associated with hemoglobin levels <13.5 g/dL in adult males and <12.0 g/dL in adult females. In patients with CKD, hemoglobin should be between 11 and 12 g/dL, however hemoglobin targets greater than 13 g/dL may increase the risk for serious adverse effects and are not recommended.^{5,10}

The KDOQI modified the EBPG definition defining anemia in adult males as hemoglobin <13.5 g/dL regardless of age since the decrease in hemoglobin levels among over 60-year-old males is frequently related to concurrent diseases. In addition, in adult females the hemoglobin target is 12 g/dL. The European Renal Best Practice (ERBP) Work Group agrees with the KDOQI definitions.¹¹

Recently, the Kidney Disease: Improving Global Outcome (KDIGO) group defined anemia in adults and children aged >15 years with CKD when the hemoglobin levels are <13.0 g/dL in males and <12.0 g/dL in females.¹² Table 1 shows the definitions of anemia and hemoglobin targets in CKD patients.

Although there is no consensus about the definition for rHuEPO resistance, the evaluation of resistance is recommended if there is an increase $\geq 25\%$ in erythropoietin dose or <1 mg/dL gain in hemoglobin levels after 2–4 weeks of treatment.¹³

According to the Brazilian Ministry of Health,¹⁴ rHuEPO resistance is defined as a persistent anemia (hemoglobin <10–12 g/dL) or the necessity of very high erythropoietin doses of epoetin alfa (300 IU/kg/week subcutaneously or 450 IU/kg/week intravenously). Epoetin alfa should be initiated at a dose of 50–100 IU/kg subcutaneously, one to three times a week. The initial goal of treatment is to achieve a rate of weekly increase in hemoglobin levels of 0.3 g/dL. If after four weeks of

treatment, this response is not observed and the hemoglobin remains below 11 g/dL, the dose should be increased by 25%. However, after four weeks if the hemoglobin level is greater than 13 g/dL, the drug should be suspended temporarily, since the maintenance of higher hemoglobin levels is associated with increased morbidity and mortality. The recommended therapeutic target is to preserve hemoglobin levels from 11 to 12 g/dL or hematocrit from 33% to 36%.⁵

Anemia in CKD is usually normocytic and normochromic. The characteristics of erythrocytes as determined by hematimetric indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) can characterize the etiology of anemia.¹⁵ In addition to hematimetric indices, the laboratory investigation includes complete blood cell count, reticulocyte count, serum iron, determination of the transferrin saturation and serum ferritin, as well as occult blood in stools and the levels of folic acid and vitamin B12.^{12,16}

The biochemical markers of iron deficiency (serum iron, ferritin, transferrin saturation and soluble transferrin receptor – sTfR) have limited value in functional iron deficiency as they are changed in several clinical conditions such as the ones that evolve with rHuEPO therapy.¹⁷ However, reticulocyte hemoglobin content (CHr or Ret-He) is a sensitive indirect marker of iron deficiency, which reflects recent changes in erythropoiesis.¹⁸ The measurement of CHr in peripheral blood samples is useful for assessing the amount of functional iron that was available in the bone marrow for new red blood cell production over the previous 3–4 days.¹⁹ CHr may be a more sensitive marker of functional iron deficiency in patients receiving erythropoietin therapy.²⁰ It may also be an early indicator of the effectiveness of iron replacement therapy.²¹

Thomas and Thomas²² presented a novel approach to functional iron deficiency with the use of CHr and the percentage of hypochromic erythrocytes (HYPO). Functional iron deficiency was defined as a CHr <28 pg and a HYPO <5% based on the levels in healthy controls. Moreover, the sTfR-F index (sTfR/log ferritin), which reflects the iron store status, can be used to differentiate functional iron deficiency in states of iron depletion and iron repletion. A diagnostic strategy combined CHr and sTfR-F index to identify four major categories of iron deficiency: (1) iron repletion with normal erythropoiesis (CHr and

Table 2 – Risk factors of resistance to recombinant human erythropoietin.

Absolute or functional iron deficiency
Gastrointestinal blood loss
Hemolysis
Inflammation
Infection
Neoplastic diseases
Malnutrition
Folic acid and vitamin B12 deficiencies
Inadequate dialysis
Hyperparathyroidism
ACE inhibitors and ARBs
Anti-erythropoietin antibodies
Genetic polymorphisms

ACE: angiotensin-converting enzyme; ARBs: angiotensin II type 1 receptor blockers.

sTfR-F index within appropriate reference values); (2) reduced iron supply with still-normal erythropoiesis (CHr within reference values; increased sTfR-F index); (3) depletion of stores and functional iron deficiency (reduced CHr; increased sTfR-F index); and (4) functional iron deficiency in a state of iron repletion (reduced CHr; sTfR-F index within reference values).

The strong association between anemia and cardiovascular complications should be highlighted. The decrease in tissue oxygenation causes tachycardia, vasodilation and increased cardiac work and may cause left ventricular hypertrophy.²³ The development and persistence of anemia in patients with CKD are also associated with worse quality of life, reduced exercise capacity, decreased mental agility and renal function and increase the prevalence of hospitalization and mortality.¹³

The main causes of resistance to treatment with rHuEPO (Table 2) in dialysis patients are discussed in this review.

Causes of resistance to treatment with recombinant human erythropoietin in patients under dialysis

Iron deficiency

Iron deficiency or impairment of iron availability is the most frequent cause of rHuEPO treatment resistance in patients under dialysis.^{24,25}

In these patients, the deficiency or reduction of total iron stores can occur due to an increase in demand of this nutrient during the production of red blood cells in the bone marrow. This absolute iron deficiency may also be related to the dialysis procedure, which promotes premature destruction of red blood cells (hemolysis), but also due to gastrointestinal bleeding, or frequent laboratory blood tests and surgeries, which patients can be submitted to.²⁶

In functional iron deficiency, suitable stores of this nutrient can be observed, but the mobilization of iron to the bloodstream is insufficient to reach the demand of the erythroid marrow. This condition is common in inflammatory states due to the cytokines that block the release of iron from deposits.²⁷

Iron deficiency anemia is characterized by microcytosis and hypochromia and a careful microscopic examination of

erythrocytes may lead to suspicion of iron deficiency. Furthermore, transferrin saturation and serum ferritin levels may help to distinguish between conditions associated with deficiency or impairment in the availability of iron.⁵

Hepcidin, a small peptide synthesized mainly in hepatocytes, is the central regulator of systemic iron homeostasis.^{28,29} Hepcidin binds to ferroportin, an iron transporter present on cells of the intestinal duodenum, macrophages, and cells of the placenta, and regulates iron release to the plasma. When hepcidin concentrations are low, molecules of ferroportin are exposed on the plasmatic membrane and release iron. When hepcidin levels increase, hepcidin binds to ferroportin inducing its internalization and degradation, thereby leading to reductions in iron release.^{30,31}

Hepcidin concentration in turn is regulated by iron, erythropoietic activity and inflammation.³² IL-6 induces hypoferremia during inflammation by inducing the synthesis of hepcidin caused by decreases in serum iron and transferrin saturation. In addition, this cytokine by itself rapidly induces hypoferremia. Since the process of erythropoiesis is the largest consumer of iron in the body, the decrease in iron supply reduces hemoglobin synthesis and can lead to anemia.³³

Hepcidin deficiency or resistance to hepcidin is associated with iron overload in hereditary hemochromatosis, iron-loading anemia, and hepatitis C. Hepcidin excess or ferroportin deficiency is the cause of iron-refractory iron deficiency anemia, ferroportin disease, anemia of inflammation, CKDs, and cancer.³⁴

Chronic inflammation and infection

The role of inflammation in the development of anemia in patients with CKD should be highlighted. It is known that the release of cytokines such as interferon-gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), interleukin 1 (IL-1) and interleukin 6 (IL-6) can induce rHuEPO erythroid progenitor cell resistance or impair the release of stored iron in the reticuloendothelial system for the production of hemoglobin.³⁵⁻³⁷ Infectious diseases may also be related to anemia resulting in chronic inflammation.³⁸

A relationship between cytomegalovirus (CMV) infection and lack of response to rHuEPO has been associated to increased production of proinflammatory cytokines such as IFN- γ and TNF- α .^{39,40} Betjes et al.⁴¹ showed an association between CMV and low hemoglobin levels in patients with CKD undergoing hemodialysis and high doses of rHuEPO.

Besides chronic inflammatory process, human parvovirus B19 (B19) can also cause anemia due to infection and lysis of erythroid precursors in the bone marrow.^{42,43} This infection may worsen in patients who have increased red blood cell destruction, as observed in patients undergoing dialysis treatment. Moreover, these patients are under increased risk of contracting HIV infection through blood transfusion or hemodialysis.⁴⁴⁻⁴⁷

Although some cases of B19 infection in patients undergoing dialysis have been reported, there are few studies which evaluated the incidence and clinical significance of B19 infection in these patients.⁴⁸⁻⁵⁰ Generally, this viral infection has been associated with transient aplastic crisis and unresponsiveness to treatment with rHuEPO.^{46,51} However, several

studies have reported the occurrence of acquired pure red cell aplasia (PRCA) and severe transfusion-dependent anemia in kidney transplant patients infected by B19.^{48,52-55} PRCA is a disorder characterized by anemia that leads to the almost complete absence of erythroid cells from precursors in the bone marrow but with normal production of granulocytes and megakaryocytes. Besides B19 infection, which has tropism for erythroid precursors in the bone marrow, other conditions that result in PRCA include the presence of autoantibodies directed against red lineage progenitors, transient erythroblastopenia in childhood, pregnancy, leukemia, infectious processes, toxins, and the use of some drugs.⁵⁶

Cofactor deficiency and malnutrition

In patients under dialysis, the main causes of protein energy malnutrition include low intake of nutrients; muscle loss due to increased protein catabolism and decrease in their synthesis; insulin resistance; loss of nutrients by dialysis and oxidative stress.⁵⁷ The inflammatory process is also a major cause of protein energy malnutrition, which may occur in 13–51% of patients under hemodialysis.⁵⁸

Malnutrition has been associated with resistance to treatment with rHuEPO in patients under dialysis. Laboratory tests show low percentages of transferrin saturation index, low serum albumin concentrations and body mass index (BMI), but high levels of C-reactive protein (CRP) in these patients.^{59,60} In addition to be a marker of iron stores, ferritin may also be increased in malnutrition.⁶¹

Deficiencies of folic acid and vitamin B12 may be associated with anemia and resistance to treatment with rHuEPO. Thus, when macrocytosis is detected, the levels of these nutrients should be evaluated.⁶² Besides the changes in erythropoiesis, folic acid and vitamin B12 deficiencies can lead to increases in homocysteine levels, which in turn is associated with an increased risk of cardiovascular complications in renal patients.^{63,64}

Inadequate dialysis

In patients with CKD, damage to erythrocytes can occur in the presence of uremic toxins, which also inhibit the production of EPO and erythropoiesis. Furthermore, the dialysis procedure causes mechanical damage to erythrocytes, and leads to blood loss.^{65,66}

The inadequacy of the dialysis dose is an important cause of anemia in patients under dialysis. In order to evaluate whether the dialysis procedure is removing enough uremic toxins, the patient's blood is sampled at the start and at the end of dialysis. The levels of urea in the two blood samples are then compared. In the Kt/V method, the dialyzer urea clearance (K) is multiplied by dialysis time (t), and the product divided by the patient's urea distribution volume (V). According to the NKF/KDOQI guidelines for patients under hemodialysis, the Kt/V target is ≥ 1.3 , and in patients under peritoneal dialysis the target is ≥ 1.7 /week.⁶⁷ A study by Gaweda et al.⁶¹ showed that patients with adequate dialysis assessed by Kt/V, require lower doses of rHuEPO. Although the pathophysiological mechanism that links inadequate dialysis to the lack of response to rHuEPO is still not completely

understood, other factors such as inflammation and vascular access complications may be associated with poorer response to treatment.

The adequacy of the dose of dialysis is also related to a decrease in costs, since patients with the best values of Kt/V require smaller doses of rHuEPO.⁶⁸

Hyperparathyroidism

Hyperparathyroidism, characterized by increased parathyroid hormone (PTH), is associated with lack of response to treatment with rHuEPO due to endogenous EPO inhibition, reduction of erythroid precursors in the bone marrow and erythrocyte survival. This hormone is also associated to the induction of bone marrow fibrosis.^{60,69,70}

According to the NKF/KDOQI,⁷¹ PTH levels between 150 and 300 pg/mL are desirable in patients undergoing dialysis. However, the threshold at which PTH levels could affect the response to rHuEPO remains unclear. Rao et al.⁷² demonstrated that patients who responded to treatment with rHuEPO had lower PTH levels (around 266 ± 322 pg/mL) compared with those who did not respond to treatment, with mean levels of 800 ± 248 pg/mL. Another study by Gaweda et al.⁶¹ demonstrated that PTH levels of 300, 600 and 900 pg/mL were associated with approximately 90%, 79% and 67% of the maximum response to treatment with rHuEPO, respectively.

Angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers

The renin-angiotensin system was previously only thought to affect the cardiovascular system. However, this system plays also an important role in hematopoiesis which explains the reduction in hematocrit levels or anemia as a side effect of treatment using angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin II type 1 receptor blockers (ARBs).^{73,74}

The ACE, which plays a central role in blood pressure control system,⁷⁵ is also responsible for the hydrolysis of acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), a tetrapeptide which naturally occurs in many body tissues. The physiological AcSDKP is a negative regulator of erythropoiesis that inhibits the entry of hematopoietic stem cells in the S phase of the cell cycle, keeping them in phase G0.^{76,77} Studies have shown that the use of ACE inhibitors is associated with increased plasma concentrations of this tetrapeptide. Thus, patients taking antihypertensive ACE inhibitors may be resistant to treatment with rHuEPO.^{78,79} The lack of angiotensin II production, due to an interruption of the renin-angiotensin system, is a direct cause of anemia, indicating that angiotensin II regulates hematopoiesis.⁸⁰ Angiotensin II acts as a growth factor and directly stimulates proliferation of erythroid progenitors in the bone marrow. Additionally, angiotensin II enhances EPO secretion, which results in increased red blood cell mass.⁷³

Decreases in hemoglobin levels occur in adults with CKD after therapy with ACE inhibitors and/or ARBs.^{81,82} These drugs have been associated with a dose-dependent decrease in hematocrit and anemia and should be considered in the differential diagnosis of anemia in patients with a variety of

illnesses including renal transplantation, decreased kidney function and heart failure. Since this effect can be reversible, the decision to decrease the dose or discontinue ACE inhibitors or ARBs therapy should consider the severity of the clinical condition and availability of alternative therapies.⁸³

Anti-erythropoietin antibodies

Although treatment with rHuEPO is well tolerated by most patients, a small number produce antibodies that can neutralize either endogenous EPO and recombinant proteins.⁸⁴ Most cases of antibody production have been associated with the formulation of epoetin alfa when administered subcutaneously.⁸⁵

In some cases, the anti-erythropoietin (anti-EPO) antibody production can lead to development of serious PRCA and transfusion-dependent anemia.⁸⁶⁻⁸⁸ Recent studies have shown that anti-EPO antibody-mediated PRCA is a rare but important adverse effect in patients with CKD who take rHuEPO.⁸⁹⁻⁹¹

According to the National Guidelines published by Brazilian Ministry of Health, PRCA should be evaluated in patients receiving epoetin alfa over at least four weeks that develop: (1) a drop in hemoglobin levels equal to or greater than 0.5 g/dL per week, in the absence of transfusions, and requirement of at least one red blood cell unit per week to maintain hemoglobin levels; (2) normal leukocyte and platelet counts; (3) absolute reticulocyte count $<10 \times 10^3/\mu\text{L}$.⁹² Treatment recommendations for patients with PRCA induced by erythropoiesis stimulating agents (ESA) are: (1) discontinuation of ESA; (2) correction of anemia by blood transfusion, if necessary; (3) kidney transplant and (4) introduction of immunosuppressive therapy starting with cyclosporine A alone or in combination with corticosteroids or corticosteroids with cyclophosphamide.^{85,86}

Diagnostic confirmation of PRCA induced by anti-EPO antibodies should include the antibody laboratory detection and a bone marrow examination that shows the absence of erythroid lineage precursors.⁹³ However, to date, there is no consensus about the method of choice for the detection of these antibodies, since the different methods have advantages and disadvantages.⁸⁷ Moreover, no commercial laboratory kit is available for the detection of anti-EPO antibodies in the clinical practice.⁸⁹

Genetic polymorphisms and the EPO receptor

Some genetic polymorphisms may result in individual response variations to rHuEPO. Jeong et al.⁹⁴ investigated the association between the interleukin 1B (IL-1B) gene and ACE gene polymorphisms and erythropoietin resistance index (RI-EPO) in patients undergoing hemodialysis. Associations between the presence of the IL-1B-511C/C and ACE D/D genotypes with lower RI-EPO were identified, indicating that these polymorphisms may be useful genetic markers in assessing the required dose of rHuEPO in patients undergoing hemodialysis.

It is known that endogenous and recombinant EPO stimulates erythropoiesis by binding to the EpoR receptor.^{95,96} An mRNA alternative splicing can give rise to the soluble form of the receptor (sEpoR) which lacks the transmembrane domain.

sEpoR has a higher affinity for EPO and acts as a potent antagonist of the hormone, which can lead to resistance to treatment with rHuEPO. Therefore, high levels of sEpoR may be associated with administration of high doses of rHuEPO.^{97,98}

Conclusion

The main cause of inadequate response to treatment with rHuEPO is iron deficiency. However, several other factors may be associated with this resistance in patients with CKD on dialysis in the presence of adequate iron stores, and must be investigated including: inflammation, infection, malnutrition, inadequate dialysis and hyperparathyroidism. In addition, B19 infection, anti-EPO antibody production, and the presence of polymorphisms have been identified as possible causes of resistance to rHuEPO in dialysis patients. However, other studies in the Brazilian population, which has its own genetic characteristics, with a larger sample size should be performed to validate these factors.

Finally, as the main reason for poor response to the use of rHuEPO is iron deficiency, which causes changes in the size and color of red blood cells, the importance of monitoring patients under dialysis through a simple blood test should be emphasized, as this may reveal morphological changes such as microcytosis and hypochromia consequent to the deficiency of this nutrient. On the other hand, deficiencies of folic acid and/or vitamin B12 also lead to clinically significant morphological changes in red blood cells, such as macrocytosis. Detection of morphological changes of red blood cells may influence proper supplementation with the adequate nutrient bringing benefits to patients under hemodialysis by properly correcting the anemia.

Conflicts of interest

The authors declare no conflicts of interest.

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