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An Overview on Resistance to Erythropoietin and Anti-Erythropoietin Antibodies

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Abstract:

The main function of red cells is to transport oxygen from the lungs to the peripheral tissues. Erythropoietin controls red-cell production by stimulating the differentiation of erythroid progenitor cells in the bone marrow. The hormone originates mainly in specialized interstitial renal cells, which respond to a decrease in oxygen delivery by increasing their production of erythropoietin. As a result, iron requirements are often increased more than fivefold in dialysis patients. Most patients with chronic renal failure achieve the desired target haemoglobin (Hb) level when supplemented with relatively low doses (50–150 IU/kg/week) of recombinant human erythropoietin (epoetin) and parenteral iron (usually 1500–3000 mg/year). About a quarter of the dialysis patients, however, have a poor response and need higher doses (>200 IU/kg/week) to reach the target Hb level. This relative resistance to Erythropoietin and iron is often associated with co-morbid conditions, particularly inflammatory conditions. The inflammatory process may be acute, leading to transient resistance to Erythropoietin, or chronic, with a persistently poor response to Erythropoietin.

Keywords: Resistance, Erythropoietin, Anti-Erythropoietin Antibodies.

Introduction:

There is no consensus on the exact definition of rHuEPO resistance. However, resistance evaluation is generally recommended if there is a ≥25% increase in the erythropoietin dose or a gain of <1 mg/dL in hemoglobin levels after 2-4 weeks of treatment [1].

Another study defined rHuEPO resistance as persistent anemia (hemoglobin <10-12 g/dL) or the necessity of very high erythropoietin doses (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 [1]**.**

The initial treatment goal is to achieve a weekly hemoglobin increase of 0.3 g/dL. If, after four weeks, this response is not observed and hemoglobin remains below 11 g/dL, the dose should be increased by 25%. Conversely, if hemoglobin levels exceed 13 g/dL, the drug should be temporarily suspended, as maintaining higher hemoglobin levels is associated with increased morbidity and mortality. The recommended therapeutic target is to maintain hemoglobin levels between 11 to 12 g/dL or hematocrit from 33% to 36% [1]**.**

Large multicenter trials of erythropoietin (EPO) show that 95-98% of patients respond positively to treatment. However, a small proportion of patients exhibit either no response or an inadequate response. While

some of these patients might respond to a much higher dose of EPO, it is crucial to first identify any underlying causes of this resistance [1]**.**

Common Causes of EPO Resistance

The most frequent issue is an inadequate supply of available iron. Other hematinic deficiencies, such as vitamin B12 or folate, are less common and should be ruled out before starting treatment. Aluminum toxicity can also cause resistance to erythropoietin, but this typically requires severe levels before inhibiting hematopoiesis. High parathyroid hormone levels have been shown to inhibit erythropoiesis in vitro, though the clinical significance of these findings remains debated **[2].**

Both acute and chronic infections, as well as occult malignancies, are potent suppressors of erythropoietic activity. Several cases have been reported where these conditions have caused EPO resistance **[3].**

Investigating Poor Responses

For patients showing a poor response or loss of a previous response to EPO, it is important to investigate potential underlying causes. Common issues such as hematinic deficiency, aluminum toxicity, hemolysis, and blood loss can usually be excluded relatively easily. However, occult infections or malignancies may be more challenging to diagnose. While it is possible to override some of these causes with higher doses of EPO, excluding them should remain a priority to ensure effective treatment **[4].**

Table 1: Causes of erythropoietin resistance. [1, 3].

Causes of erythropoietin resistance
Absolute or functional iron-deficiency anemia
Hyperparathyroidism
Aluminum toxicity
Concurrent infection
Systemic inflammation (elevated acute phase proteins i.e. CRP, fibrinogen)
Vitamin B12 or folate deficiency
Hemolysis
Bone marrow disorders
Hemoglobinopathies
Carnitine deficiency
Angiotensin-converting enzyme inhibitor therapy
Anti-erythropoietin antibodies
Gender (females have less response to erythropoietin, and thus need higher doses, than do males)
Advanced age

Mode of dialysis (patients on hemodialysis need higher doses of erythropoietin than those on peritoneal dialysis)
Inadequate dialysis (decreased dose or adequacy)
Chemical or biological contamination of water used for dialysis

The primary causes of resistance to rHuEPO in dialysis patients include:

- Iron Deficiency:

Iron deficiency or impaired iron availability is the most frequent cause of rHuEPO resistance in dialysis patients. This can result from increased iron demand during red blood cell production, premature red blood cell destruction (hemolysis), gastrointestinal bleeding, frequent blood tests, and surgeries. Functional iron deficiency, characterized by adequate iron stores but insufficient mobilization to meet erythroid marrow demand, is common in inflammatory states due to cytokines blocking iron release from deposits **[5].**

- Hepcidin Regulation:

Hepcidin, a peptide synthesized mainly in hepatocytes, is the central regulator of systemic iron homeostasis. It binds to ferroportin, an iron transporter on intestinal duodenum cells, macrophages, and placental cells, regulating iron release to the plasma. Low hepcidin levels expose ferroportin on the membrane, releasing iron, while high hepcidin levels induce ferroportin internalization and degradation, reducing iron release. Hepcidin concentration is regulated by iron, erythropoietic activity, and inflammation [6].

- Chronic Inflammation and Infection:

The significant role of inflammation in precipitating anemia among children with CKD warrants attention. Cytokines like interferon-gamma, tumor necrosis factor-alpha, interleukin 1 (IL-1), and interleukin 6 (IL-6) are known to induce resistance of erythroid progenitor cells to rHuEPO or impede the release of stored iron in the reticuloendothelial system necessary for hemoglobin production **[7].**

Infectious diseases can also contribute to anemia by fostering chronic inflammation. The correlation between cytomegalovirus (CMV) infection and unresponsiveness to rHuEPO is linked to heightened production of proinflammatory cytokines like IFN-gamma and TNF.In addition, there is a relationship between CMV and reduced hemoglobin levels in CKD patients on hemodialysis, necessitating high doses of rHuEPO **[8].**

Furthermore, human parvovirus B19 (B19) infection can induce anemia by infecting and lysing erythroid precursors in the bone marrow. This infection can exacerbate in children experiencing increased destruction of red blood cells, such as those undergoing dialysis treatment. Additionally, these patients face an elevated risk of contracting HIV infection via blood transfusion or hemodialysis **[9].**

Although B19 infection cases in dialysis patients have been documented, studies evaluating the incidence and clinical significance of B19 infection in this population remain scarce. Generally, B19 infection has been associated with transient aplastic crisis and resistance to rHuEPO treatment. However, several studies have reported instances of acquired pure red cell aplasia (PRCA) and severe transfusion-dependent anemia in kidney transplant patients infected by B19 **[10].**

PRCA manifests as anemia with an almost complete absence of erythroid cells in bone marrow precursors while maintaining normal production of granulocytes and megakaryocytes. Besides B19 infection, which targets erythroid precursors in the bone marrow, other PRCA causes include autoantibodies against red lineage

progenitors, transient erythroblastopenia in childhood, pregnancy, leukemia, infections, toxins, and certain medications **[10].**

- Cofactor Deficiency and Malnutrition:

In children undergoing dialysis, protein-energy malnutrition arises from various factors such as inadequate nutrient intake, muscle wasting due to increased protein breakdown and decreased synthesis, insulin resistance, loss of nutrients during dialysis, and oxidative stress. This condition is often exacerbated by inflammation, affecting a significant proportion of patients undergoing hemodialysis **[11].**

Malnutrition has been linked to resistance to treatment with recombinant human erythropoietin (rHuEPO) in dialysis patients. Laboratory tests typically reveal low levels of transferrin saturation, serum albumin, and body mass index (BMI), alongside elevated levels of C-reactive protein (CRP) in these children. Despite being a marker of iron storage, ferritin levels may also be elevated in cases of malnutrition **[3].**

Deficiencies in folic acid and vitamin B12 are associated with anemia and resistance to rHuEPO treatment. Therefore, when macrocytosis is observed, the levels of these nutrients should be assessed. Additionally, deficiencies in folic acid and vitamin B12 can lead to elevated homocysteine levels, which are linked to an increased risk of cardiovascular complications in patients with renal disease **[12]**

- Inadequate Dialysis:

Several factors contribute to erythrocyte damage and subsequent anemia in CKD cases. Uremic toxins present in CKD can hinder the production of erythropoietin (EPO) and erythropoiesis. Additionally, the mechanical stresses of the dialysis procedure can further damage erythrocytes and lead to blood loss **[13]**.

Insufficient dialysis dosing represents a significant cause of anemia in dialysis patients. To assess the efficacy of dialysis in removing uremic toxins, blood samples are taken before and after the dialysis session, and the levels of urea in these samples are compared. The Kt/V method, a widely used measure, calculates the dialyzer urea clearance (K) multiplied by dialysis time (t), divided by the patient's urea distribution volume (V) **[14].**

According to guidelines from the National Kidney Foundation/Kidney Disease Outcomes Quality Initiative (NKF/KDOQI), the target Kt/V is ≥1.3 for patients undergoing hemodialysis and ≥1.7/week for those on peritoneal dialysis. Improving the adequacy of dialysis dosing not only enhances patient outcomes but also reduces healthcare costs, as patients with optimal Kt/V values necessitate smaller doses of rHuEPO **[14, 15].**

- Hyperparathyroidism:

Hyperparathyroidism, characterized by elevated PTH levels, is linked to a lack of response to treatment with rHuEPO due to several factors. These include inhibition of endogenous EPO, reduction of erythroid precursors in the bone marrow, and decreased survival of erythrocytes. Additionally, this hormone is associated with the development of bone marrow fibrosis **[3].**

NKF/KDOQI guidelines suggest that PTH levels between 150 and 300 pg/mL are desirable in patients undergoing dialysis. However, the exact threshold at which PTH levels might affect the response to rHuEPO remains uncertain. **Stevens et al., [15]** demonstrated that patients who responded to rHuEPO treatment had lower PTH levels (around 266 ± 322 pg/mL) compared to non-responders, who had mean levels of 800 ± 248 pg/mL **[15].**

- ACE Inhibitors and ARBs:

The renin-angiotensin system was traditionally believed to only impact the cardiovascular system. However, it also plays a significant role in hematopoiesis, explaining the reduction in hematocrit levels or anemia

observed as a side effect of treatment with angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin II type 1 receptor blockers (ARBs) **[16].**

ACE, which is central to the blood pressure control system, also hydrolyzes acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), a tetrapeptide naturally occurring in many body tissues. Physiological AcSDKP acts as a negative regulator of erythropoiesis, inhibiting the entry of hematopoietic stem cells into the S phase of the cell cycle and keeping them in phase G0. Studies have demonstrated that the use of ACE inhibitors is linked to increased plasma concentrations of this tetrapeptide **[16].**

Consequently, patients taking ACE inhibitors for hypertension may exhibit resistance to treatment with rHuEPO. Anemia directly results from the lack of angiotensin II production, due to interruption of the renin-angiotensin system, indicating that angiotensin II regulates hematopoiesis. Angiotensin II acts as a growth factor, directly stimulating the proliferation of erythroid progenitors in the bone marrow. Additionally, it enhances EPO secretion, leading to an increase in red blood cell mass **[16].**

Cases with CKD experience decreases in hemoglobin levels after therapy with ACE inhibitors and/or ARBs. These medications have been associated with a dose-dependent reduction in hematocrit and anemia, necessitating their consideration in the differential diagnosis of anemia in patients with various 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 take into account the severity of the clinical condition and the availability of alternative therapies **[17].**

- Anti-Erythropoietin Antibodies (anti-EPO):

Although most patients tolerate treatment with rHuEPO well, a small subset may develop antibodies that neutralize both endogenous EPO and recombinant proteins. The majority of cases involving antibody production have been linked to the formulation of epoetin alfa when administered subcutaneously. In some instances, the production of anti-EPO antibodies can lead to the development of serious PRCA and transfusion-dependent anemia. Recent research has highlighted that anti-EPO antibody-mediated PRCA is a rare yet significant adverse effect among CKD patients undergoing rHuEPO therapy **[18].**

According to the National Guidelines outlined by the Brazilian Ministry of Health, PRCA should be assessed in patients receiving epoetin alfa for at least four weeks who experience:

(1) a decline in hemoglobin levels equal to or greater than 0.5 g/dL per week, in the absence of transfusions, and the need for at least one unit of red blood cells per week to maintain hemoglobin levels.

(2) normal leukocyte and platelet counts.

(3) an absolute reticulocyte count of less than 10 x 103 /μL [3].

Treatment recommendations for patients with PRCA induced by erythropoiesis stimulating agents (ESA) include:

1) Discontinuation of ESA.

(2) Correction of anemia through blood transfusion if necessary.

(3) Kidney transplant.

(4) Initiation of immunosuppressive therapy, which may involve cyclosporine A alone or in combination with corticosteroids or corticosteroids with cyclophosphamide [3].

To confirm the diagnosis of PRCA induced by anti-EPO antibodies, laboratory detection of the antibodies and a bone marrow examination demonstrating the absence of erythroid lineage precursors are required. However, there is currently no consensus on the preferred method for detecting these antibodies, as different methods have their advantages and disadvantages. Furthermore, there are no commercial laboratory kits available for detecting anti-EPO antibodies in clinical practice **[19]**.

- Genetic Polymorphisms:

Certain genetic polymorphisms can influence individual responses to rHuEPO. A previous study explored the relationship between polymorphisms in the interleukin 1B (IL-1B) and ACE genes and the erythropoietin resistance index (RI-EPO) in hemodialysis patients. The study found that patients with IL-1B-511C/C and ACE D/D genotypes had lower RI-EPO, suggesting these polymorphisms could serve as genetic markers to predict the necessary dose of rHuEPO for pediatric undergoing hemodialysis **[20].**

Endogenous and recombinant EPO function by binding to the erythropoietin receptor (EpoR) to stimulate erythropoiesis. However, alternative splicing of EpoR mRNA can produce a soluble form of the receptor (sEpoR) that lacks the transmembrane domain. This sEpoR has a higher affinity for EPO and acts as a competitive antagonist, potentially leading to resistance to rHuEPO treatment. Consequently, elevated levels of sEpoR might necessitate higher doses of rHuEPO **[21].**

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