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Pattern of resistance to erythropoietin-stimulating agents in chronic kidney disease

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Routine administration of erythropoietin (EPO)-stimulating agents (ESAs) for the control of anemia has improved the quality of life of subjects with chronic kidney disease (CKD). However, a wide variation in individual response to ESA is often observed. The reasons for EPO resistance include demographic variables such as age and gender distribution, morbidity pattern, and modality of dialysis. Despite suggestions by observational data, there is no biological characteristic that puts children at a disadvantage for adequate response to ESA. On the contrary, children possess a superior capacity for red cell production, including extramedullary erythropoiesis. The reasons for larger requirement of ESA in children (than in adults) are greater inflammatory burden, disproportionate blood loss, and greater EPO dosing by pediatric physicians. To minimize the harmful (including fatal) consequences of EPO resistance, surveillance programs must replenish nutrient (for example, iron and folate) stores, minimize oxidative hemolysis, control hyperparathyroidism, avoid catheter infection, and optimize uremic clearance. This clinical approach is justified by the inadequacy of laboratory diagnosis of pertinent etiological factors. Indeed, the best proof for functional nutrient deficiency is often a therapeutic trial. Finally, there are upcoming therapeutic agents that exploit the capacity for an endogenous EPO synthesis in CKD subjects, and may therefore minimize the off-target effect of excess dosages.

Kidney International (2011) **80,** 464–474; doi:10.1038/ki.2011.179; published online 22 June 2011

KEYWORDS: chronic kidney disease; clinical nephrology; erythropoietin; inflammation

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Received 20 December 2010; revised 2 March 2011; accepted 22 March 2011; published online 22 June 2011

In the absence of standard diagnosis criteria, studies on resistance to erythropoietin (EPO)-stimulating agents (ESAs) have depended on *ad hoc* definitions. Consequently, there is inability to compare data obtained from multiple sources. Data from the United States Renal Data System showed that there is a wide variation in the dosing pattern of ESAs in the renal population.[1–4] Thus, the mean EPO doses given to the top 99th percentile are 30 times larger than the amount received by the lower 1%. Target hemoglobin (Hb) mass (11–12 g/dl) is only achieved in 90–95% of patients after treatment with 1000–30,000 IU/week of short-acting ESAs.[1–4] In general, ESA resistance is presumed in adult subjects if Hb mass is <11 g/dl (over a 4–6-month period) despite a weekly dose of EPO in excess of 500 IU/kg or 30,000 IU/week ($\geqslant 1.5$ μg/kg for darbepoietin).

However, there are insufficient data in the pediatric population that will allow generation of a similar definition. Nevertheless, according to the 2004 NAPRTCS (North American Pediatric Renal Transplant Cooperative Study) data, the average dosing requirement for EPO in pediatric subjects is 350 IU/kg.[2] The disproportionate larger doses of EPO in children are concerning and may in part reflect the extrapolation of adult-dosing regimen by pediatric physicians.[5] A more equitable dosing may be achieved using absolute Hb deficit and/or body surface area (greater correlation with blood volume) as criteria for prescription. Furthermore, there is a greater prevalence of amenable etiological factors of ESA resistance in pediatric subjects than in adults.[5] Therefore, it may be reasonable to adopt a lower threshold of EPO dosing for pediatric diagnosis. Thus, evaluation of ESA resistance should be commenced if Hb mass is <11 g/dl (over a 3-month period) in spite of a weekly dose of EPO of >400 IU/kg or $>20,000$ IU/week ($\geqslant 1.0$ μg/kg for darbepoietin).

Although standardized definition has its usefulness, care must be taken not to fall prey to its unintended consequences. We must recognize the role of poorly amenable risk factors of ESA resistance: including female menstrual status, androgen deficit in elderly males, dialysis modality, and age-related comorbidities. Hence, the rationale for an individual evaluation should be based on clinical judgment and demographic peculiarities.

Harmful effects of ESA resistance

A recent meta-analysis of studies evaluating higher Hb target with the use of ESA in chronic kidney disease (CKD) suggests

there is a greater risk for stroke, cardiovascular events, and mortality rate.[6–9] However, it is not clear whether the adverse outcomes are related to EPO dosing, higher Hb mass, or some (hidden) variables. Nevertheless, there is an undoubted need for a cautious dosing of ESA, whereas adequate knowledge of its independent effect will require randomized clinical trials.

Similarly in a geriatric cohort study, there is a correlation between higher concentration of endogenous EPO and fatal outcome.[10] Although this finding may reflect a physiological response to an undiagnosed hypoxemia, impaired bone marrow response (senescence) is not unlikely.[10] Hence, the resultant excessive EPO synthesis may promote off-target biological consequences. Furthermore, a recent study has suggested that a segment of hemodialysis (HD) population may maintain natural Hb mass in excess of 12 g/dl without a greater mortality rate.[11] Given the complexity of EPO resistance, a careful analysis of its etiology and appropriate intervention are often warranted.

ETIOLOGY OF ESA RESISTANCE

The pattern of risk factors in a dialysis program is dependent on sociodemographic variables, mode of clinical practice, and quality of renal care.[12] Methodology including the choice of diagnostic parameters often influences the outcome of the studies.

Age and gender

Despite suggestions by observational data, there is no innate biological characteristic that puts children at a disadvantage for an adequate response to ESA. On the contrary, children are known to have a superior capacity for hematopoiesis, including the use of an extramedullary organ (such as the spleen) when necessary.[13] In a recent comparative study of pediatric and adult dialysis cohorts, a ten-times larger dose of ESA was required in the younger cohort. The reason for the disparity includes greater inflammatory stress and disproportionate blood loss.[14] Furthermore, the findings of a greater need of EPO in adult females may be due to androgenic stimulation of erythropoiesis in males.[15] Similarly, women with preserved capacity for menstrual cycles, despite the hypogonadotrophic effect of CKD, often require greater amounts of EPO.[16]

Uremia and ESA resistance

Although the mechanism is uncertain, there is reduced renal capacity to synthesize EPO in chronic uremia, whereas there is blunted bone marrow response to its biological action (Table 1). Nevertheless, as a result of persistent stimulation from pervasive anemia, there is a five-fold higher than normal EPO concentration for the level of Hb deficit.[17] Finally, clinical evidence of uremic inhibition is demonstrated by the correlation of urea nitrogen clearance with improved cytokine profiles (interleukin (IL)-6, C-reactive proteins) and lower requirement for ESA.[18]

Outside of the poor bone marrow response to ESA, there is an accelerated turnover rate of red cells from persistent oxidative stress, increased osmotic fragility, and progressive depletion of adenosine triphosphate (ATP). Uremia alters erythrocyte morphology by inducing outward expression of the phosphatidyl-serine content of its inner membrane, which is removed by circulating macrophages.[19,20] In addition, there is impaired cellular generation of ATP by uremic inhibition of pentose phosphate shunt and tricarboxylic acid cycle.[21,22] These cells, which are depleted of ATP, manifest poor capacity for cytosolic calcium efflux and thereby inhibit mitochondrial oxidative phosphorylation, thus, setting up a vicious cycle.[23,24]

Oxidative stress, anemia, and ESA response

Oxidative stress promotes ESA resistance by causing lipid peroxidation of red cell membranes.[25] It depletes the capacity for ATP generation by interfering with mitochondrial function (Table 1). Uremia in turn enhances cellular oxidation by increasing the activity of superoxide dismutase.[26] Furthermore, renal retention of asymmetric dimethyl arginine, and low cellular content of L-arginine analog, inhibits synthesis of its nitric oxide metabolite.[27] The magnitude of (uremic)

Table 1 | The risk factors, pathogeneses, and therapeutic modulation of erythropoietin resistance in pediatric and adult subjects with chronic kidney disease

Risk factors	Mechanism of ESA resistance	Therapeutic intervention
Uremic toxins[a,b]	↓EPO synthesis/↓erythroid response	Longer effective dialysis
Oxidative stress[a,b]	Downregulation of HIF	Vit E and vit C
Inflammation[a,b,c]	Cytokines: IL-1, IL-6, TNF-α	Avoid sepsis and malnutrition
Iron deficiency[a,c]	Hemoglobin synthesis	Replenish iron/↓ blood loss
Hyperparathyroidism[a,c]	Vitamin D synergism (erythropoiesis)	Low P diet/ 1,25 OH vit D
Aluminum toxicity[a,c]	Aluminum bone disease	Avoid aluminum intake
Hemolysis[a,c]	Uremia/HbSS/G6PDD/AIHA	Uremic clearance/vit E and C
Drugs: angiotensin-modulating agents[a,b]	↓Erythroid ANG II receptors/↑endogenous EPO inhibitor, AcSDKP	↓Dose of ACEi/ARB

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; AcSDKP, *N*-acetyl-seryl-aspartyl-lysyl-proline; AIHA, autoimmune hemolytic anemia; ANG, angiotensin; ARB, angiotensin receptor blocker; EPO, erythropoietin; ESA, EPO-stimulating agent; G6PDD, glucose 6-phosphatase deficiency; HbSS, hemoglobinopathies; HD, hemodialysis; HIF, hypoxic-inducible factor; IL, interleukin; TNF-α, tumor necrosis factor-α; vit, vitamin.
[a]Adult data; applicable to both populations.
[b]Experimental data; applicable to both populations.
[c]Pediatric and adult data.

oxidative burden is made worse by the reduction in erythrocyte glutathione, an anti-oxidant tripeptide, by extracorporeal exposure to dialyzer membranes.[28]

The pro-oxidative renal tissue downregulates the generation of hypoxic-inducible factor-1 alpha (HIF-1α) protein, thereby reducing EPO synthesis and red cell production.[29] This contributes to pervasive anemia, which in turn, increases the fragility of erythrocyte by membrane (lipid) peroxidation.[25] Therefore, to break the vicious cycle, correction of anemia seems like a cost-effective strategy.

EPO is a pleiotropic hormone that is crucial for anemia control and suppression of oxidative stress by upregulation of heme oxygenase-1.[30] It protects the animal model of ischemia–reperfusion by activation of the cell survival signal pathways.[31] However, adjunct therapy with iron elaborates free oxygen radicals and may potentially reverse the anti-oxidative benefit of ESA.[32,33]

Inflammation and ESA resistance

Careful data analysis often suggests that persistent inflammation is a confounding variable in fatality associated with CKD. Contributory factors are bio-incompatible dialyzers, dialysate (water) contamination, long duration of HD catheters, poor dialysis, and previous allograft rejection (Table 1).

Cytokines and ESA. Uremic toxins are implicated in the propagation of proinflammatory cytokines: IL-1, IL-6, interferon-γ, and tumor necrosis factor.[34,35] These cytokines downregulate the expression of EPO receptors on erythroid progenitors and disrupt iron recycling by blocking its release from reticuloendothelial cells (Figure 1).[36]

In normal circumstances, macrophages ingest the senescent erythrocyte and release its iron contents into the lysosomes. Ferrous ions are then transported across the cell membrane by ferroportin carrier protein. The latter is inhibited by hepcidin, an acute-phase reactant that is synthesized by the liver after stimulation by IL-6.[37] Iron is transported in the plasma by the transferrin carrier protein, which enhances its cellular uptake by binding to its surface receptors.[38] Cytokines may also upregulate iron cellular uptake by activating divalent metal transporter and/or transferrin.[38]

Mechanism of EPO receptor activation. EPO binds to erythroid cell by spontaneous dimerization of its surface receptors (Figure 2). The persistent activation allows for interaction with receptor even at a low EPO concentration.[39] Resistance to ESA may result from defective dimerization of EPO receptors. Similarly, activation of the EPO receptor is prevented by (antagonistic) a mimetic peptide such as cytokine-inducible SH2-containing protein.[40]

After binding to its receptor, EPO activates JAK2 tyrosine kinase, which in turns promotes intracellular phosphorylation leading to initiation of a signal transduction.[40] In addition, by regulating the activation of EPO receptors, tyrosine phosphatase (SHP-1) desphophorylates JAK2.[41] Hence, there is serum elevation of SHP-1 in HD subjects with ESA resistance.[41]

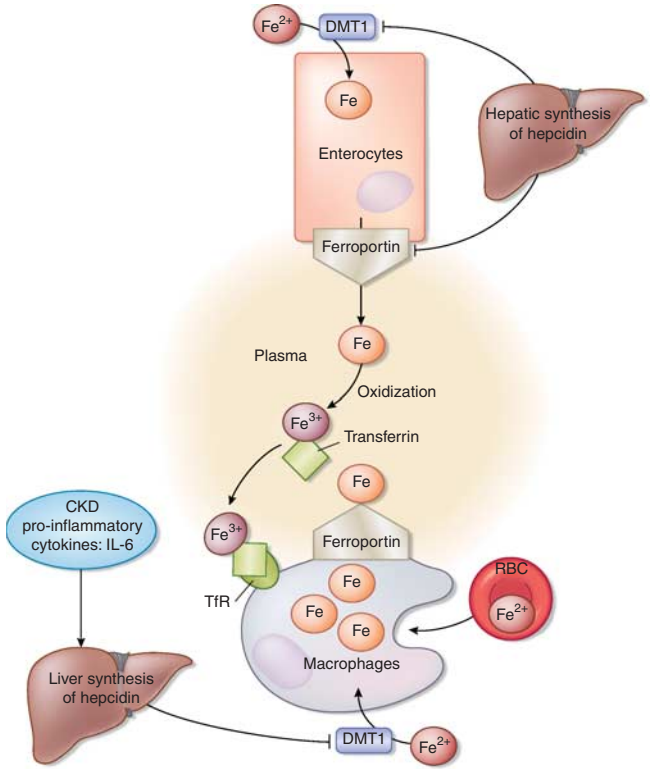

Figure 1 | Schematic illustration of erythropoietin receptor activation and intracellular signal transduction. CKD, chronic kidney disease; DMT1, divalent methyl transporter-1; RBC, red blood cell; TfR, transferrin receptor.

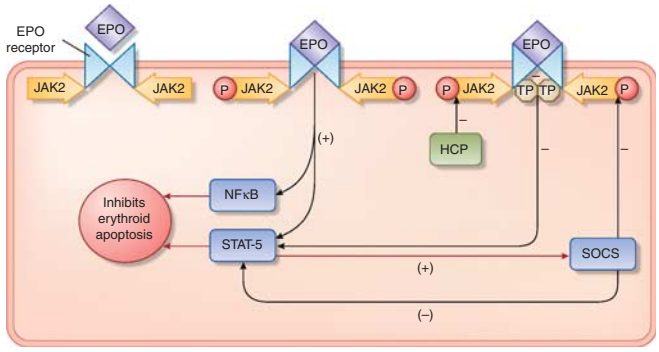

Figure 2 | Process of physiological iron recycling involving macrophages and enterocytes. EPO, erythropoietin; HCP, hematopoietic cell phosphatase; NFκB, nuclear factor κB; JAK2, Janus kinase 2; P, phosphorylation; TP, tyrosine phosphatase; SOCS, suppressor of cytokine signaling; STAT-5, signal transducer and activator of transcription-5; − (minus sign), inhibition; + (plus sign), activation.

In response to the activation of intracellular domain of the EPO receptor, both signal transducer and activator of transcription-5 (STAT-5) and nuclear factor κB are phosphorylated. These pathways then upregulate mitochondrial expression of (anti-apoptotic) Bcl-xl and Bcl-2 proteins, thereby promoting erythroid cell proliferation.[42,43] In addition, STAT-5 participates in negative regulatory feedback and induces suppressor of cytokine signaling (or cytokine-inducible SH2-containing protein), which in turn downregulates

signal transduction by preventing JAK2 phosphorylation of the EPO receptor.[44] Similarly, hematopoietic cell phosphatase inactivates JAK2. Suppressor of cytokine signaling may also inhibit EPO effect by a direct inactivation of STAT-5. Proinflammatory cytokines promote EPO resistance by activation of suppressor of cytokine signaling and subsequent inhibition of nuclear factor κB.[44–46]

Failed allograft and ESA. It is estimated that 4.5% of ~10,000 subjects on maintenance dialysis in the United States, between the years 1996 and 2001, have had at least one failed allograft. In spite of the larger EPO requirement in this sub-group, there is 50% higher incidence of anemia, greater decline in kidney function, and a lower survival rate compared with controls.[47] If the etiological role of failed allograft is suspected, therapeutic care must be individualized. The risk of transplant nephrectomy must be carefully weighed against the clinical benefits. In the events of symptoms of transplant rejection, persistent elevation of inflammatory indices, and intractable anemia despite large doses of ESA, nephrectomy is a reasonable approach.[48]

Dialysis catheters. Additional sources of oxidative inflammation are the use of perm-cath and synthetic grafts as vascular access in HD subjects.[49] Even in the absence of infection, compared with arteriovenous fistula, HD catheters and synthetic arteriovenous grafts promote systemic inflammation.[50,51] Compared with subjects using native arteriovenous fistulae, larger doses of effective ESA and greater need for blood transfusion are observed in those dialyzed with using artificial vascular access.[51] Furthermore, routine use of anti-platelet prophylaxis may aggravate iron deficiency among patients dialyzed with AV grafts.

Finally, to minimize the adverse impact of inflammation, optimal control of uremic toxicity is necessary. In this regard, nocturnal HD has been shown to enhance clearance of middle molecules, suppress inflammatory (IL-6) cytokines, and thereby reduces ESA requirement.[52]

Iron deficiency and ESA resistance

Iron deficiency, principally due to poor nutrition, is not only common in CKD but is also most probably a universal occurrence in dialysis subjects (Table 1; Figure 3). There is an estimated annual loss of 2 *g* of elemental Fe among adult subjects on maintenance HD.[53] Aggravating iron deficiency in end-stage kidney disease is the depletion of tissue store by the supra-physiological proliferation of erythroid cells that results from ESA therapy.[54] As a result of frequent blood sampling, smaller body mass, higher rate of catheter use, and greater incidence of infection, pediatric subjects are more susceptible to iron deficiency.[12] Additional source of iron loss is uremic gastritis; its incidence may be potentiated by a concomitant infection with *Helicobacter pylori*.[55]

There is good evidence that suggest that adjunct use of intravenous iron with ESA is a cost-effective strategy for anemia control in chronic HD.[56] For this reason, routine evaluation of iron repletion is often necessary. Serum iron and transferrin saturation provide a modest but effective means of assessing iron bioavailability, whereas adequacy of tissue stores can be estimated with total iron-binding capacity and ferritin. Low serum ferritin is an early indicator of iron deficiency, but a high serum value may be confounded by inflammatory status. Therefore, a minimum of two indices of serum iron (<100 mg/dl), transferrin saturation (<20%), and serum ferritin (<100 ng/dl) is required to make a diagnosis.[57–59] Although oral iron is used as prophylaxis, because of superior efficacy, parenteral route is frequently required for adequate repletion. To avoid systemic overload during inflammation process, parenteral iron therapy is withheld if serum ferritin level exceeds 500–800 mg/dl,

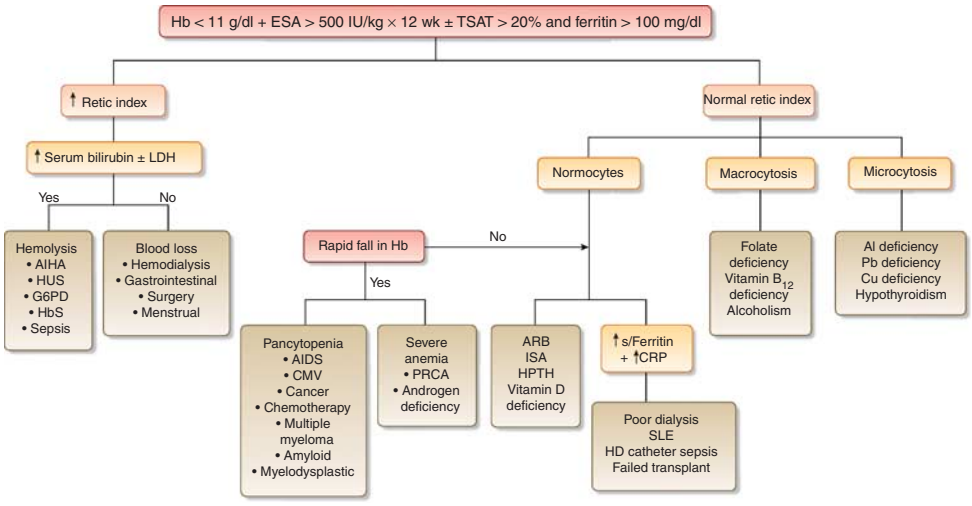

Figure 3 | Schema for clinical evaluation of resistance to erythropoietin stimulating agent in chronic kidney disease. AIDS, acquired immunodeficiency syndrome; AIHA, autoimmune hemolytic anemia; CMV, cytomegalovirus; CRP, C-reactive protein; ESA, erythropoietin-stimulating agent; G6PD, glucose 6-phosphate dehydrogenase deficiency; Hb, hemoglobin; HD, hemodialysis; HPTH, hyperparathyroidism; HUS, hemolytic uremia syndrome; ISA, immunosuppressive agents; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; PRCA, pure red cell aplasia; SLE, systemic lupus erythematosis; TSAT, transferrin saturation.

whereas vitamin C could be used to mobilize iron from reticuloendothelial stores.[57–59]

Secondary hyperparathyroidism

Suggesting an etiological role of hyperparathyroidism, there is a higher prevalence of anemia and greater EPO requirement among HD subjects who are in the upper 50th percentile of intact parathyroid hormone.[60,61] As a proof of causal relationship, surgical parathyroidectomy led to an improved control of anemia and a lower need for ESA.[62] Nevertheless, there is no evidence for a direct inhibition of erythropoiesis by excessive PTH. Neither is there a proof of associated bone marrow fibrosis in experimental animals.[63] On the contrary, there is a positive correlation between serum PTH and systemic mobilization of progenitor cells in primary hyperparathyroidism.[64]

Resistant anemia is more likely a result of complex interaction of uremic toxins, PTH, and inflammatory cytokines (Table 1). An evidence for this assertion is the downregulation of bone cell expression of PTH, transforming growth factor-β, and insulin-like growth factor-1 receptors in uremia, whereas there is altered response to IL-1, IL-6, and tumor necrosis factor-α cytokines.[65] In addition, metabolic acidosis and hyperphosphatemia may underlie the resistance to ESA in uremic bone disease. Both conditions downregulate EPO receptors by causing a rightward shift of the oxygen–Hb dissociation curve. This theory was supported by a regression analysis that showed a correlation between ESA resistance and hyperphosphatemia, despite an adequate control of PTH effect.[66]

Consequently, effective strategy for the control of ESA resistance in metabolic bone disease must encompass dialysis restoration of non-uremic homeostasis. In addition, therapeutic modulation of uremic bone morphology may be possible in the near future. Thus, treatment of uremic animal with bone morphogenetic protein-7 not only maintained the osteoblastic phenotype but also prevented bone marrow fibrosis.[67]

1,25-vitamin D deficiency

Aggravating the capacity to synthesize the active form of vitamin D in CKD is the inadequate exogenous supply of 25-OH vitamin D (Table 1; Figure 3). Low serum 25-(OH) vitamin D (<65 nmol/l) occurs in 80% of subjects with glomerular filtration rate of 20–90 ml/min per 1.73 m^2. Reasons for deficiency include poor sunlight exposure, deficient skin synthesis, low dietary intake, and nephrotic urinary losses.[68–70] In addition to the role of vitamin D in bone mineral metabolism, pleiotropy is suggested by a wide receptor distribution and its synergistic effect on ESA control of anemia.[71] In a similar manner, calcitriol treatment in subjects with uremic bone disease increases the proliferation of erythroid precursors.[72] Corroborating the evidence for synergism is a lower EPO requirement among subjects with BB polymorphism of vitamin D receptor genotype compared with those with the heterozygous *Bb/bb* gene.[73,74]

Heavy metal

Subjects with stage 4–6 CKD are at risk for toxicity from heavy metal because the renal route is the principal mode of elimination (Table 1; Figure 3). Bone marrow function is often impaired in individuals with heavy metal poisoning.[75] Aluminum (Al) toxicity is a rare event in modern practice because of discontinuation of its use as a phosphate binder, and a lower Al content in dialysis water. However, inadvertent intake of Al-containing compounds may occur in (developing) countries with poor control of pharmaceutical standards.[75] Heavy metal exposure (cadmium, lead, and mercury) may result from industrial pollution. Similarly, the use of alternative herbal supplements is not uncommon even among the educated elites. Some folk remedies of Indian and Middle Eastern origins are enriched with heavy metals (rasa shastra) based on a presumed therapeutic efficacy.[76]

Furthermore, close to 16% of children inhabitants of cities with a population greater than one million, who reside in homes built before 1946, have elevated lead levels.[77] Although there are no available data, lead toxicity is more likely to occur in patients with CKD. This is because both iron and calcium deficiencies, commonly seen in CKD, increase the gastrointestinal absorption of heavy metals.[78] In addition, uremia may increase mobilization of lead from bone tissue stores. In general, lead toxicity causes anemia by precluding incorporation of iron into a protoporphyrin ring for heme synthesis.[78]

Hemoglobinopathy and hemolytic anemia

Anemia in CKD subjects, perpetuated by comorbid hematological disorders (B-thal, HbS, HbA2, HbH), is frequently associated with elevated endogenous EPO (Figure 3).[79] The most common hemoglobinopathy is sickle cell disease. It occurs more frequently in individuals of African, Mediterranean, South American, Asian, and Middle Eastern descent.[79–81] The mechanism of EPO resistance includes bone marrow infarction, red cell hemolysis, hypersplenism, and ineffective erythropoiesis.[79,80] As newborn screening is mandated in most developed economies, most patients with hemoglobinopathies are identified before the onset of CKD.[82] In addition, the recent trend of improving quality of care (with a longer survival) and insidious onset of renal consequence make end-stage kidney disease an unlikely event in pediatric subjects, while prevalence in adult program is expected to increase.[82,83]

In view of its low prevalence, data on the impact of hemoglobinopathy on EPO-resistant anemia are rare. In a recent Italian study of HD subjects with β-thalassemia minor, correlation was found between higher doses of ESA and plasma content of Hb A2.[84] Therefore, it is desirable to consider the role of Hb disease in ESA resistance in endemic (geographical) area that lacks newborn screening programs. Even in the developed world, there should be awareness of its confounding effect among immigrant subjects and/or those with enhanced ethnic susceptibility.

Subjects with chronic uremia and hemolytic diseases are more susceptible to the morbid impact of oxidative stress,

nutrient deficiency, and dialysis blood loss (Table 1). Common chronic hemolytic conditions are sickle cell disease, thalassemia, hereditary spherocytosis, glucose 6-phosphate dehydrogenase deficiency, and auto-immune diseases.[85] Similar to hemoglobinopathy, there is ethnic susceptibility to glucose 6-phosphate dehydrogenase deficiency. It occurs in 13% of Black males and in 2% of Black females.[85]

In subjects with CKD that is preceded by systemic lupus erythematosis and hemolytic uremia syndrome, relapses must be evaluated in the event of intractable anemia (Figure 3). Autoimmune hemolytic anemia occurs in 5–10% of patients with systemic lupus erythematosis; it is frequently associated with renal or neurological involvement.[86–88] As reticulocytosis is common in patients on ESA therapy, early diagnosis of hemolytic events may be missed. It should be suspected when there is a progressive increase in EPO requirement while there is rapidly decreasing Hb concentration. An additional clue to autoimmune process in systemic lupus erythematosis is the concurrent depression of megakaryocytic cell line. Serum titer of anti-double-stranded DNA is often elevated.[88]

Unlike the more common diarrhea-associated hemolytic uremia syndrome, the atypical form is often more severe, relentless, and frequent.[89] Its etiology may include the absence of regulatory inhibition of complement C3 activity, which results from hereditary factor H deficiency. Similarly, deficiency of von Willebrand factor cleaving-protease activity predisposes to recurrent micro-angiopathic episodes. Both events may be temporarily controlled by infusion of fresh frozen plasma. Furthermore, transplant hemolytic uremia syndrome may occur (*de novo*) particularly among patients exposed to calcineurin inhibitors, those who had bone marrow transplant and/or acute viral infection.[89]

Angiotensin-modulating agents

The influence of angiotensin-converting enzyme inhibitor/angiotensin receptor blockers on ESA resistance is controversial. A range of studies, mostly observational, have either suggested an etiological role or dismissed that there is a causal association (Table 1). A prototype study showed there is a higher EPO requirement in dialysis patients who were treated for hypertension with either angiotensin-converting enzyme inhibitors or angiotensin receptor blockers compared with those placed on calcium channel blockers.[90] A genetic mechanism for angiotensin modulation is suggested by a favorable response to ESA in dialysis subjects with insertion/deletion ACE polymorphism.[91] Furthermore, angiotensin-converting enzyme inhibitor suppresses the enzymatic degradation of *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), a naturally occurring inhibitor of erythropoiesis.[92] Similarly, stimulation of erythroid cellular proliferation by angiotensin binding of its type II surface receptor is inhibited by angiotensin receptor blockers.[93] This forms the basis for the therapeutic use of angiotensin receptor blockers in post-transplant erythrocytosis.

Route of administration

The subcutaneous route is considered a more effective means of ESA delivery; it requires lower EPO dose than does the intravenous route for the same biological effect.[94] However, a follow-up study showed no disparity in EPO requirement for either subcutaneous or intravenous route if there is adequate Fe repletion.[95] The convenient use of intravenous therapy makes it a preferred modality in HD programs, whereas self-administered subcutaneous route is used in peritoneal dialysis patients and non-dialysis CKD.

Non-iron micronutrients

The role of non-iron micronutrient deficiency in ESA resistance is often underestimated (Figure 3).[96] The extraordinary ESA stimulation of bone marrow, high catabolism, and losses with frequent dialysis are predisposing factors for nutrient deficiency. The poor bioavailability of hematopoietic nutrients (folate, vitamins B_{12} and C) could limit the efficiency for erythropoiesis. Micronutrient deficiency is a potential contributory factor for greater mortality in subjects that failed to attain Hb target despite large doses of EPO.[7–9] Thus, in a longitudinal dialysis cohort, serum deficiency of water-soluble vitamins (B_1, B_6, C, and folates) had resulted from a substantial loss in 50% of patients. Lower amounts of lipophilic vitamins (A and E) were measured in the dialysates.[97]

Folate deficiency

Although folate deficiency is a rare finding in studies that depend on serum sample for diagnosis, a greater EPO requirement was observed in a pediatric dialysis cohort that had hematological response to a therapeutic trial.[96] Laboratory assay of serum folate is prone to error because of its poor prediction of tissue stores.[98–100] Etiologies of folate deficiency in end-stage kidney disease may include poor gastrointestinal absorption, poor diet, water-soluble nutrient loss, and high catabolic rate.[101–103] Folic acid is an essential ingredient for nucleotide synthesis, DNA repair, and re-methylation of homocysteine. Owing to its requirement for excessive erythroid proliferation, adequate supplementation is mandatory for an optimal effect of ESA.[104] Furthermore, FD produces oxidative vascular injury by potentiating uremic inhibition of homocysteine catabolism.[105]

Vitamin C deficiency

Unlike other animals, human subjects solely depend on dietary source for ascorbic acid, as it lacks the capacity for its endogenous synthesis. Vitamin C is a cofactor for several enzymatic metabolism.[106] Owing to its water solubility, there is a greater loss of vitamin C in subjects undergoing hemodiafiltration compared with those on peritoneal dialysis.[101,107,108] Furthermore, an increased risk for vitamin C deficiency occurs in a sub-population (of Asian) with haptoglobin gene polymorphism (hp 2-2).[109]

In addition, ascorbic acid promotes gastrointestinal absorption of iron and enhances its mobilization from tissue

stores. It increases Hb synthesis by facilitating incorporation of Fe into protoporphyrins. Vitamin C, an anti-oxidative free oxygen scavenger, downregulates hepatic synthesis of cytokines.[110–113] Its intravenous use in HD subjects reduces serum level of ferritin, an inflammatory biomarker.[110–113] As symptoms of vitamin C deficiency (namely anemia, asthenia, myalgia) are similar with the findings in CKD, early diagnosis is often jeopardized.[111,112] Therefore, its deficiency must be considered in the event of persistent anemia that failed to respond to EPO and Fe supplements.

Circulating EPO inhibitors

Resistance to biological effects of EPO may result from its neutralization by a circulating antibody or by its inactivation by binding the soluble plasma receptors (Figure 3).[114] Pure red cell aplasia is a rare cause of anemia that results from antibody neutralization of EPO. It was seen with higher frequency (191 events) in Europe between 1998 and 2002. It could have resulted from a substitution of polysorbate-80 emulsifier for human albumin in the formulation of ESA. Its immunogenicity was enhanced by subcutaneous administration and certain host biological factors. Incidence of pure red cell aplasia decreased with the removal of offending EPO (Eprex) from the European market.[115]

Pure red cell aplasia should be suspected in a patient who had received ESA therapy for >4 weeks and had experienced a rapid decrease in Hb mass (>0.5 g/dl per week), reduction in absolute reticulocyte count $<10,000$ per μl, and/or >1 Unit per week of red cell transfusion.[116–118] Non-erythroid cell lines including leucocytes and platelets are normal. The more common causes of EPO-resistant anemia should be excluded. There is an absence of erythroid precursors on bone marrow sampling and low EPO content of the serum. The serum sample inhibits growth of erythroid colonies in a bone marrow culture. Radioimmunoassay identifies circulating neutralizing anti-EPO IgG.[119] Discontinuation of rhEPO will result in rapid fall of the neutralizing antibody. Steroid and/or calcineurin inhibitor may be successful.[120] Poor response may warrant plasma exchange and/or allograft transplantation. Routine blood transfusion should be avoided except for life-threatening anemia. Hematide, a pegylated synthetic peptide, which is immunologically distinct from EPO, has been used to minimize cross-reactivity.[121]

POTENTIAL PHARMACOLOGICAL INTERVENTION IN EPO RESISTANCE

Although rarely used, certain aspects of EPO resistance may be amenable to drug intervention. Thus, there are ongoing clinical trials on anti-oxidative agents, nutritional supplements, and stimulants of endogenous erythropoiesis.

Anti-inflammatory agents

Delivery of vitamin E, either by impregnation of HD filters or by its oral therapy, reduces oxidative hemolysis that results from chronic dialysis. Similarly, α-tocopherol protects vitamin A, ascorbic acid, and polyunsaturated fatty acids from inactivation by free radicals.[122,123] Furthermore, there is an ongoing European clinical trial on the efficacy of Oxpentifylline (a xanthine derivative) as an inhibitor of inflammatory cytokines (IL-1 and tumor necrosis factor-α) among dialysis subjects.[124,125] Similarly, by improving cytokine profiles, anti-lipemic agents promote responses to EPO, an effect that may occur even in the absence of hyperlipidemia.[126]

Nutritional supplements

To maintain an efficient erythropoiesis, there must be adequate supply of essential hemopoietic nutrients. These include iron, folic acid, copper, vitamin C, vitamin B_{12}, α-lipoic acid, and levocarnitine. α-lipoic acid is a coenzyme of mitochondrial dehydrogenase that is required for the synthesis of ATP. It suppresses oxidative stress, in part by depleting serum concentration of symmetric-dimethyl arginine.[127] Similarly, L-carnitine promotes efficient utilization of cellular energy by enhancing mitochondrial transfer of fatty acid. It stimulates heme oxygenase-1, an antioxidant that shares metabolic pathway with EPO.[128] Carnitine deficiency may result from excess catabolism and losses from dialysis procedures. Consequently, its intravenous replenishment is associated with lower EPO requirement, lesser oxidative biomarkers, and positive nitrogen balance.[129–131]

Long-acting EPO

Concern for the adverse effects of excessive EPO has led to the synthesis of product with larger molecular weight and longer elimination half-life. Thus, a more efficient erythropoiesis (and lower side effects) is promoted by prevention of wide oscillation in biological activity that characterizes the short-acting variant.

Darbepoietin-α. Using insertion mutagenesis to increase the *N*-linked glycosylated (bonding) sites for sialic acid residues, ESA with a larger molecular weight was synthesized. Its elimination half-life was increased by more than threefold. Thus, a weekly subcutaneous dose of darbepoietin effectively corrects anemia in most CKD subjects.[132,133] Unlike short-acting EPO, its use in cancer patients did not result in disease progression or lower survival rate.[133]

Continuous EPO receptor activator. Continuous EPO receptor activator was synthesized by integration of EPO protein with a polyethylene (glycol) polymer, thereby increasing its half-life to 130 h. Anemia was successfully controlled after a twice monthly regimen in a recent phase III clinical trial.[132]

EPO fusion with human IgG. Additional strategy aimed at increasing EPO potency is the genetic fusion of its N terminal with the Fc region of human IgG. It allows binding of the (aerosolized) EPO conjugate with Fc receptors on epithelial lung surfaces and thereby facilitates its active transportation into the systemic circulation.[134] The feasibility of its clinical application was recently shown by a successful conduct of a phase I human trial.[132]

EPO-mimetic peptide. Pegylated peptide (Hematide) differs from EPO in its homology sequence, but has greater (1000-fold) affinity for its receptor and therefore has a longer biological half-life. Owing to its low immunogenicity, it is used as rescue therapy in EPO antibody-mediated red cell aplasia.[132]

Endogenous induction of EPO

Furthermore, the potential for avoiding morbidities from excessive EPO (and/or EPO resistance) is more likely with strategies that exploit the capacity for endogenous EPO production.

Prolyl hydroxylase inhibition (HIF stabilizers). HIF is a transcriptional complex that regulates EPO gene expression by oxygen.[132] In iron-replete cells, its α-subunits are degraded by prolyl hydroxylase, a process that requires 2-oxoglutarate as a cofactor. Therefore, analog substitution of the cofactor with N-oxalylglycine (HIF stabilizers) activates transcription of EPO synthesis. A unique advantage of this agent is its capacity for an oral administration. In addition, HIF stabilizers facilitate iron recycling by upregulating (hypoxia-sensitive) genes for EPO receptors, transferrin, and ferroportin.[135] However, there is a credible concern that coactivation of gene for vascular endothelial growth factor may promote an off-target development of oncogenesis.[135]

Erythroid survival mediators. The protein product of *growth arrest-specific gene 6* (*Gas6*) mediates the survival of erythroid cell lines. ESA stimulates erythroblast for the release of *Gas6*, which in turn induces serine-threonine kinase (Akt) for signal activation of the EPO receptor.[136] Therefore, adjunct therapy with *Gas6* gene by-product may allow lower therapeutic doses of ESA. Unlike rhEPO, Gas6 produces no adverse effect of erythrocytosis.[136] Similarly, *IL-3* regulates gene transcription (Gata-2 and Scl) for survival of immature erythroid cells, whereas EPO increases (Gata-1) mRNA and protein stability.[137]

EPO gene therapy. A successful clinical application of gene therapy lies in the ability to purify and deliver the genetic material while maintaining safety, cost efficacy, and biological potency. The recent use of human mesenchymal stem cells, as a vector for the *hEPO* gene in a mouse model, results in a life-long control of Hb deficit, holding promise for its use in human population.[138] Given that stem cells are immune privileged, a convenient intramuscular delivery every 2–4 times per year is feasible. Similar to endogenous EPO, coupling of the gene with a hypoxia-responsive DNA element may allow oxygen-dependent regulatory feedback.[139] A potentially viable and cost-effective technique for gene therapy is the molecular engineering of viral proteins with the hope of circumventing the natural barriers to its (viral) survival.[140]

In summary, the tremendous success recorded with the use of EPO for control of anemia of CKD in the last two decades is recently tempered by the concern for its safety when administered at higher doses. Surveillance program for early detection EPO resistance and strategies to minimize their biological impact must be built into all renal programs. Owing to the challenges in establishing diagnosis, often the best proof for hemopoietic nutrient deficiency is a therapeutic trial. Consequently, empirical supplementations with Fe and similar nutrients are imperative. Finally, there are upcoming therapeutic agents that exploit the capacity for endogenous EPO synthesis in CKD subjects; and may hopefully minimize the off-target effects of excess dosages.

DISCLOSURE

The author declared no competing interests.

ACKNOWLEDGMENTS

I thank Dr M Vehaskari and Dr D Aviles of Louisiana State University Health Science Center, New Orleans for their editorial assistance.

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