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Markers of Iron Status in Chronic Kidney Disease Diagnosing Iron Deficiency

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Abstract

Anemia is one of the main comorbidities related to Chronic Kidney Disease (CKD). Until the advent of Erythropoiesis Stimulating Agents (ESA), endogenous erythropoietin deficiency has been thought to be the main culprit of anemia in CKD patients. The use of ESA's has shed new light on the physiology of CKD anemia, where iron homeostasis plays an increasingly important role. Disorders of iron homeostasis occurring in CKD turn the anemia management in those patients into a complex multifactorial therapeutic task, where ESA and Iron dose must be properly balanced to achieve the desired outcome without exposing the patients to the risk of serious adverse events. This review covers diagnostic markers traditionally used for quantifying iron status in CKD patients, such as serum ferritin and transferrin saturation, new ones, such as reticulocyte hemoglobin content and percent hypochromic red cells, as well as experimental ones, such as hepcidin and soluble transferrin receptor. Each marker is presented in terms of their diagnostic performance, followed by biological and analytical variability data. Advantages and disadvantages of each marker are briefly discussed. Although serum ferritin and transferrin saturation are easily available, they exhibit large biological variability and require caution when used for diagnosing iron status in CKD patients. Reticulocyte hemoglobin content and the percentage of hypochromic red cells are more powerful, but their widespread use is hampered by the issue of sample stability in storage. Soluble transferrin receptor and hepcidin show promise, but require further investigation as well as the development of standardized, low-cost assay platforms.

Introduction

Anemia is one of the most common comorbidities of Chronic Kidney Disease (CKD) and is precipitated by multiple mechanisms including but not limited to: 1) decline in endogenous erythropoietin production in kidneys[1, 2], 2) shortened erythrocyte survival[3–5] 3) nutritional deficiencies (folate, vitamin B12)[6, 7]. The introduction of recombinant human erythropoietin (rHuEpo) revolutionized the treatment of anemia in patients with advanced CKD[8], whose primary form of treatment up to that point consisted of repeated blood transfusions potentially leading to secondary iron overload[9]. Although the use of rHuEpo freed patients from transfusion dependence and reduced the risk of iron overload, it brought to light another important role that this metal plays in the anemia of CKD[10]. Being the oxygen

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carrier while at the same time having the ability to damage cells through free radicals, systemic iron must be tightly balanced[11].

In healthy individuals, iron is recycled from the senescent erythrocytes and transported via the reticuloendothelial system into the bone marrow where it is incorporated into erythroblasts[1]. Normal iron homeostasis is maintained by compensating daily loss through duodenal absorption of dietary iron[12]. Due to increased blood losses, especially in dialysis dependent patients[13], as well as potentially compromised gastrointestinal iron absorption [14–16], the total body iron stores in CKD patients are likely to be depleted resulting in *absolute iron deficiency*. In addition, many CKD patients suffer from *functional iron deficiency*, defined as the inability to deliver sufficient iron amount to the site of erythroblast production, even in presence of adequate stores, typically due to reticuloendothelial cell iron blockade. Due to the multifactorial nature, proper quantification of the iron status in CKD patients is challenging.

Methods of Iron Quantification

Existing clinical practice guidelines focus on measuring iron status using two standard indices: 1) serum ferritin and 2) transferrin saturation[17].

Serum Ferritin

Ferritin is the main iron storage protein whose production is regulated by the intracellular iron concentration. Each ferritin molecule has a capacity to store more than 4000 iron atoms[18]. Analysis of ferritin iron content has revealed that despite elevated ferritin concentration, hemodialysis patients might have a decreased ferritin iron content[19]. In addition to functioning as iron storage, ferritin is also regulated by acute phase proteins [20, 21].

Ferritin concentration is commonly used as the primary index for diagnosing absolute iron deficiency. Whereas low levels of ferritin concentration (<100 ng/dL – non-dialysis, <200ng/dL – dialysis-dependent) enable physician to reliably diagnose absolute iron deficiency, there exists no consensus as to the upper ferritin limit in dialysis dependent CKD patients[22–24]. Retrospective analyses of the erythropoietic response previously showed that elevated ferritin concentrations might predict erythropoietic hyporesponsiveness[25, 26], potentially caused by the malnutrition inflammation complex syndrome[27]. On the other hand, a steady rise in average ferritin levels that has been observed among dialysis patient population in the United States has been attributed to the increase in intravenous iron utilization and the decrease in Erythropoiesis Stimulating Agents (ESA) use [28].

Several studies analyzed biological and analytical variability of serum ferritin[29, 30]. Both studies concluded that due to large biological variability, single measurements of serum ferritin had limited diagnostic value in evaluating iron status in CKD patients and should not be used to guide clinical decisions regarding treatment of iron deficiency. In clinical practice, serum ferritin is measured at quarterly intervals in both non-dialysis and dialysis dependent CKD patients. To improve the diagnostic performance of serum ferritin, a more frequent measurement schedule might be necessary.

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Transferrin Saturation

Plasma transferrin is a glycoprotein with two iron-binding domains and is synthesized by the liver[31]. It is the most important vehicle for transporting iron into cells and preventing iron-mediated free radical toxicity. Approximately 3 mg of iron (0.1% of total body iron stores) circulates in the plasma and is bound to transferrin. The sum of all iron binding sites on plasma transferrin is known as Total Iron Binding Capacity (TIBC). Transferrin Saturation (TSat) is the ratio of total number of occupied iron binding sites to TIBC. The number of available iron binding sites is known as Unsaturated Iron Binding Capacity (UIBC). In healthy individuals, approximately one-third of the iron binding sites are occupied (TSat ≈ 33 %) [32]. When the total iron-binding capacity of transferrin is exceeded, Non-Transferrin Bound Iron (NTBI) is produced. NTBI is virtually undetectable until TSat reaches 80%[33].

In the care of CKD patients, low TSat (< 20%) combined with low serum ferritin is diagnostic of absolute iron deficiency. Low TSat combined with normal or elevated serum ferritin is diagnostic of functional iron deficiency. Retrospective analyses of erythropoietic responsiveness showed that maximum erythropoietic response is achieved at TSat levels greater than 30%[25, 26, 34]. Iron repletion / supplementation protocols used by most dialysis organizations in the United States specify TSat target range 30-50%.

Biological and analytical variability of TSat and serum iron has been studied repeatedly[29, 35–38]. All studies consistently report remarkable biological variability of TSat (as high as 38%[29]). Although some of this variability could be partially explained by diurnal changes in serum iron concentration[39], these results call for caution when using TSat to quantify iron status in CKD patients. Similar to serum ferritin, a more frequent measurement schedule might be required to improve the diagnostic performance.

Other readily available laboratory parameters can be used to aid iron status quantification in CKD patients[40]. These include: 1) reticulocyte hemoglobin content (equivalent), 2) percentage hypochromic red cells.

Reticulocyte Hemoglobin Content (Equivalent)

Under normal erythropoietic conditions, young erythrocytes (reticulocytes) are released from the bone marrow into circulation and become mature erythrocytes after several days. Reticulocyte Hemoglobin Content (CHr), first introduced in Siemens analyzers, quantifies hemoglobin mass in reticulocytes, providing information about a short-term change in iron status. In a study of 78 patients undergoing bone marrow examination, CHr proved superior to serum ferritin and TSat in diagnosing iron status in the bone marrow[41]. In patients with pre-existing diagnosis of functional iron deficiency, CHr turned out to be a better predictor of response to intravenous iron treatment compared to serum ferritin and TSat[42]. CHr is approved both in United States and Europe as marker of functional iron deficiency with a diagnostic threshold of 29 pg.

Reticulocyte Hemoglobin Equivalent (RetHe), introduced by Sysmex, is an alternative measure of hemoglobin mass in reticulocytes. Multiple studies found strong correlation between RetHe and CHr in iron deficient CKD patients[43, 44]. In a study of 504 subjects with different anemia etiologies, a diagnostic threshold of 25 pg was established to identify iron

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deficiency (<25 pg) from other forms of anemia[44]. Furthermore, in a study of 40 dialysis patients, Urrechaga et al.[45] found RetHe to be a reliable predictor of response to intravenous iron supplementation at a diagnostic threshold of 30.8 pg.

Van Wyck et al. [29] demonstrated that biological and analytical variability of CHr was significantly smaller than that of serum ferritin and TSat. When measuring CHr (RetHe) in clinical practice, the time delay between blood sample draw and analysis should be taken into account when interpreting the results. Dialysis organizations in the United States typically use centralized laboratories with specimen shipment times ranging between 24 to 48 hours. Due to the quick maturation process of reticulocytes, there is a risk that this time delay may introduce measurement bias. A study of sample stability by Lippi et al. [46] showed that there was a small but statistically significant decrease in CHr values when samples were stored for 24 hours in 4 C.

A study of 60 newly diagnosed elderly anemic patients compared the utility of CHr and Mean Cellular Hemoglobin (MCH) in screening for iron deficiency using bone marrow stain as a gold standard[47]. Both markers showed comparable diagnostic performance at thresholds of 30.5 pg (CHr) and 28.5 pg (MCH), respectively. This finding suggests that MCH, which is usually reported as part of a standard Complete Blood Count panel, may be a useful alternative for diagnosing iron deficiency when CHr or RetHe are not available. CHr (RetHe) provide information about short-term trends and may be useful for monitoring early response to ESA/Iron dose changes. On the other hand, MCH provides information about long-term trends and may be more useful during maintenance therapy.

Percentage Hypochromic Red Cells

Hypochromic red cells (HRC) are erythrocytes with Mean Cellular Hemoglobin Concentration (MCHC) less than 28g/dL. The clinical utility of %HRC in diagnosing functional iron deficiency was first described by Macdougall et al. [48]. With a diagnostic threshold of 6 %, %HRC was found to be superior to other iron indices in detection of iron deficiency[49]. Similar to CHr, %HRC was first introduced on Siemens analyzers. Sysmex introduced another measure of hypochromia, %Hypo-He, which defines red cells with MCH < 17 pg. In a previously mentioned study, Urrechaga et al. demonstrated the reliability of %Hypo-He in predicting response to iron supplementation at a diagnostic threshold of 2.4%[45]. Several other studies demonstrate the utility of %HRC and %Hypo-He in diagnosing iron deficiency and predicting response to intravenous iron treatment in CKD patients[50–52].

Because measurement of %HRC and %Hypo-He takes into account the size of the erythrocytes, the clinical utility of this test is somewhat compromised when samples are shipped to centralized laboratories as is the case in the United States, due to the fact that erythrocytes may expand in storage[40].

In addition to above mentioned laboratory parameters, commercially available on automated analyzers, there are other parameters that may shed more light on the pathophysiology of iron deficiency component of CKD anemia: 1) soluble transferrin receptor, 2) hepcidin.

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Soluble Transferrin Receptor

Iron uptake into erythroid precursors occurs through internalizing transferrin bound iron. The transferrin bound iron binds to transferrin receptors present on the surface of the plasma membrane and is internalized through endosomes ultimately leading to the release of iron. Plasma membrane bound transferrin receptors are released into the blood in form of a truncated soluble transferrin receptor (sTfR). Iron-restricted erythropoiesis may cause overexpression of sTfR, as increased number of transferrin receptors may come off the surface of the erythroblasts and become detectable[53–55].

Soluble transferrin receptor has been demonstrated to be superior to serum ferritin in differentiating between iron deficiency anemia and anemia of inflammation[56]. However, in CKD patients receiving ESA treatment, sTfR appears to be more of a marker of erythropoietic activity itself than iron-restricted erythropoiesis[57, 58]. Evaluation of sTfR as a predictor of response to intravenous iron treatment in dialysis dependent CKD patients on ESA showed that at a diagnostic threshold of 1.5mg/L, sTfR was superior to serum ferritin and TSat, but inferior to %HRC and CHr[49]. A measure combining sTfR and serum ferritin (sTfR index), calculated as the ratio of sTfR to log-transformed serum ferritin [59] was evaluated in 121 dialysis-dependent CKD patients and at a diagnostic threshold value of 0.6 proved to be superior to serum ferritin and TSat in predicting response to intranvenous iron treatment[60]. In a study of 145 patients with iron deficiency anemia or anemia of chronic disease, Skikne et al.[61] showed that the sTfR index was superior to sTfR alone in differentiating between the two anemia etiologies. They also showed a doubling in detection rate in iron deficiency anemia when all serum ferritin, sTfR, and sTfR index were used together, compared to using ferritin alone. Although further studies might be needed to shed more light on the diagnostic utility of sTfR, currently available evidence suggests that this marker is best used in combination with other iron indices. Clinical utility of sTfR as a measure of iron status in CKD patients is further compromised by the cost of the assay and the lack of standardization.

Hepcidin

Hepcidin is a peptide produced by the liver to regulate iron absorption and mobilization[62]. Hepcidin level is affected by iron stores, inflammation states, and erythropoiesis[63, 64]. These factors, as well as decreased renal clearance are believed to be the main determinants of hepcidin levels in CKD patients[65].

In a study of 20 dialysis-dependent CKD patients, there was a significant short-term reduction of serum hepcidin after dialysis treatment [66]. Hepcidin concentration was further positively correlated with serum ferritin and TSat, negatively correlated with reticulocyte count, and not correlated to inflammatory cytokines. Hepcidin concentration has been demonstrated to decrease following administration of ESA administration in some studies[63, 67]. In a study of 56 dialysis-dependent CDK patients, Tessitore et al.[68] showed that serum hepcidin concentration was not an important predictor of a response to intravenous iron treatment in patients receiving ESA. A similar conclusion was reached by the FIND-CKD investigators[69]. Further studies are ongoing to determine whether and how hepcidin measurement can be used to quantify iron status in patients with CKD anemia. An

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assessment of biological hepcidin variability over 2- to 6-week period suggests that short-term measurement of hepcidin might not be useful for guiding clinical decisions regarding iron status in CKD patients[70]. The potential clinical utility of hepcidin as a marker of iron status is further complicated by the lack of consensus on a unified methodology and standards to measure hepcidin.

Conclusion

Anemia in CKD patients is a multifactorial complex problem influenced by the combination of insufficient erythropoietin production, absolute and function iron deficiency, as well as chronic inflammatory states. As of now, a nephrologist treating anemia has a choice diagnostic tools at their disposal to discern the anemia etiology. While serum ferritin and transferrin saturation have been the most commonly used laboratory parameters due to their wide availability, both these markers are subject to excessive biological variability and require caution when used for guiding iron supplementation in CKD patients. Cellular indices of iron status, such as reticulocyte hemoglobin content or percentage of hypochromic red cells, based on the iron content measurement in newly created erythrocytes, are more powerful in diagnosing anemia as well as guiding the treatment. Their widespread use is hampered by the issue of sample stability in storage. Newly available parameters, such as soluble transferrin receptor and hepcidin show promise, but require further investigation to establish the exact way in which they can be used in diagnosing the etiology of CKD anemia. For future use in the clinical practice, these new markers will also require development of standardized, low-cost assay platforms.

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