NEW INSIGHTS INTO IRON METABOLISM AND DEFICIENCY

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MEETING SUMMARY

The symposium provided an overview of the prevalence of iron deficiency and the associated disease burden in patients with chronic kidney disease (CKD). Prof Kai-Uwe Eckardt gave an overview of the prevalence of iron deficiency in patients with CKD not undergoing dialysis and addressed the challenge of diagnosing iron deficiency in this patient population based on the definitions currently used. Prof Tomas Ganz then reviewed the pathophysiology of iron metabolism, and explained the complex interplay of hepcidin in making iron available for erythropoiesis. The symposium concluded with a presentation from Prof Jolanta Małyszko who reviewed the methods of determining iron status among patients with CKD and compared data on the benefits and risks of intravenous (IV) and oral iron therapy.

Prevalence of Iron Deficiency in Patients with Chronic Kidney Disease: A Matter of Definition?

Professor Kai-Uwe Eckardt

The three main causes of renal anaemia are erythropoietin (EPO) deficiency, iron deficiency, and inflammation. Although iron deficiency is a cause of renal anaemia, it can also manifest in other ways. Diagnostic methods of measuring tissue iron content include bone marrow biopsy (an invasive procedure) and liver magnetic resonance imaging, which is not routinely available. The use of surrogate markers such as ferritin and the transferrin saturation (TSAT) as diagnostic tools is routine clinical practice, although they come with several limitations, including being influenced by

the presence of inflammation. In the general population, the threshold for the normal levels of these surrogate markers is lower than if measured in a population of patients with CKD on dialysis, but the appropriate cut-offs for patients with CKD not on dialysis (ND-CKD) is less clear. A recent systematic review concluded that guidelines[1] recommend the use of higher thresholds of ferritin and TSAT in patients with ND-CKD. When looking at a range of interventional iron studies in patients with ND-CKD, the inclusion criteria for iron parameters also stipulate higher threshold values for ferritin and TSAT. However, when using such high threshold values in patients with ND-CKD, the frequency of 'iron deficiency' in this patient population is high. An analysis of data from the National Health and Nutrition Examination Survey (NHANES) found that the majority of patients with CKD had levels of serum ferritin <100 ng/mL or TSAT <20%.[2]

The German Chronic Kidney Disease (GCKD) study is an observational prospective cohort study that aims to increase the understanding of the natural course of CKD, by identifying and validating the risk factors and markers for the manifestation, progression, and complications of CKD. This study recruited over 5,000 patients[3] and an analysis of iron parameters revealed that the majority of patients do not meet the target levels established in CKD haemodialysis patients. Whether this indicates a high prevalence of iron deficiency or impropriety of such cut-off values in patients with less advanced CKD, is difficult to define. In conclusion, diagnosis of iron deficiency in patients with ND-CKD remains a challenge. The risk-benefit relationship for treatment of these patients rather than specific laboratory values should guide therapy.

Iron Pathophysiology: Its Complexity and Our Knowledge Gaps

Professor Tomas Ganz

There are two kinds of iron regulation in the body. The first is systemic regulation, whereby the organism regulates its dietary iron absorption, the concentration of iron in extracellular fluid, and iron storage. The second is cellular regulation, whereby iron uptake and subcellular distribution are controlled at the level of each individual cell.

Erythrocytes are made in the bone marrow and contain iron; each millilitre of packed erythrocytes represents a milligram of iron. The lifecycle of an erythrocyte is 110–120 days, after which it is taken up by macrophages in the spleen and liver, and the iron is transferred to the plasma, where it binds to transferrin and circulates until it is taken up by the bone marrow again to make more erythrocytes (Figure 1).

Due to the lack of excretory mechanisms, very little iron is normally lost from the body; however, in patients with CKD and those on dialysis this loss is increased. The usual homeostatic processes ensure that increased losses in iron are compensated, either by increased absorption of iron in the small intestine and duodenum, or the use of iron from the liver where surplus iron is stored. These compensatory mechanisms are greatly affected during infection and inflammation, leading to a reduction in plasma iron concentrations known as 'hypoferraemia of inflammation', eventually leading to the development of anaemia due to a reduced production of erythrocytes.

Erythropoietic stimulation, a process that results in the production of more erythroid precursors involved in the generation of erythrocytes, requires additional iron to be absorbed into the duodenum, or taken up from hepatocytes into the plasma.

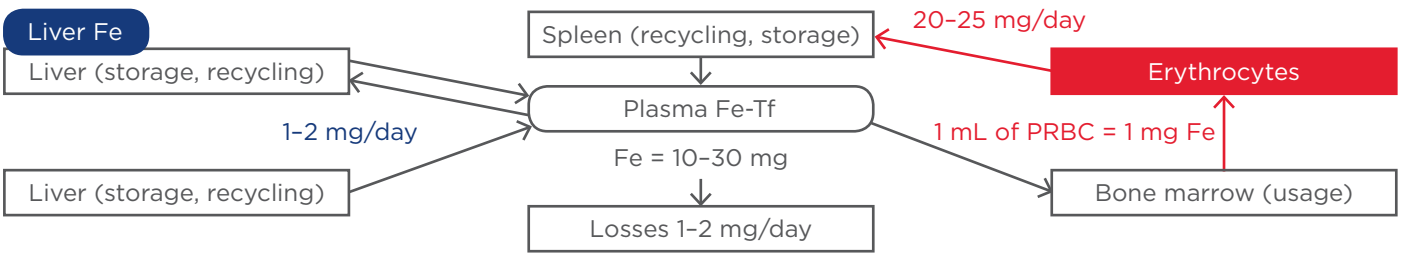


Figure 1: Overview of systemic iron metabolism.
Fe: iron; PRBC: packed red blood cells; Tf: transferrin.

This is a normal physiological process and often occurs during bleeding or if EPO is administered. The chief regulator of iron homeostasis is hepcidin, which is secreted by hepatocytes as the 84-amino-acid preprohepcidin and then cleaved to a 25-amino-acid bioactive hepcidin by the prohormone convertase furin.[4] Hepcidin regulates intestinal iron absorption and iron distribution in tissues by binding to the ferroportin receptor, a 12-transmembrane-segment protein that is present in macrophages, in the duodenum, on hepatocytes, and in the placenta.[5-7] Binding of hepcidin to the ferroportin receptor results in its degradation[8] and decreased cellular iron export. When hepcidin is low, duodenal enterocytes absorb dietary iron and export it into the blood, but high hepcidin inhibits these processes. Thus hepcidin regulates dietary iron absorption and the influx of iron to the plasma at the level of iron absorption, and also similarly at the level of iron recycling and at the level of release from stores.

Hepcidin levels are regulated by levels of iron in the plasma, iron stores in the liver, and erythropoietic signals from the bone marrow. Administration of iron and subsequent measurement of hepcidin levels have shown that, in response to iron, there is a spike in serum iron with an increase in serum and urinary hepcidin.[9] Detection of plasma iron takes place via a complex of transferrin receptors (transferrins 1 and 2) and a human haemochromatosis molecule on the external membrane of hepatocytes that senses the concentration of holo-transferrin and conveys this message intracellularly, resulting in increased hepcidin messenger ribonucleic acid (mRNA) and consequently increased hepcidin production.

Intestinal iron absorption is greatly increased after the administration of EPO and in forms of anaemia in which erythropoiesis is active, such as non-transfused β-thalassaemia.[10] These observations suggest that there is a circulating factor that connects erythropoiesis to iron regulation, and that this factor is likely produced in the bone marrow. One study of five male volunteers who were given EPO has shown that serum hepcidin levels drop 9–24 hours after administration and this effect lasts for at least 5 days, with minor reductions in transferrin, ferritin, and levels of the transferrin receptor.[11] Searches for the factor connecting erythropoiesis to iron regulation has led to the identification of erythroferrone, which is highly expressed in EPO-stimulated erythroblasts and acts as an erythroid regulator of iron metabolism. During anaemia or hypoxia, the kidneys produce EPO which stimulates erythroferrone production and in turn suppresses the production of hepcidin in the liver, increasing iron absorption and making more iron available for erythropoiesis.

During infection or inflammation, hepcidin levels increase and serum iron levels decrease, as demonstrated in an *in vivo* human endotoxaemia model.[12] By contrast, in hepcidin knockout mice that were given an inflammatory stimulus, increased levels of iron were evident,[13] demonstrating that hypoferraemia of inflammation is dependent on hepcidin. Patients with CKD have hepcidin-dependent anaemia, hepcidin-independent anaemia, and a relative lack of EPO. Hepcidin-dependent effects in these patients are mediated by an increase in inflammatory cytokines (e.g. interleukin [IL]-6) that increase hepcidin and cause iron trapping in macrophages, resulting in a reduction in available iron and the restriction of haem and haemoglobin synthesis and erythropoiesis. Hepcidin-independent effects in patients with CKD include shortened erythrocyte lifespan and the direct suppression of erythropoiesis by cytokines. In patients with progressive CKD, levels of hepcidin were higher and directly proportional to the severity of kidney disease, compared with paediatric or adult controls.[14,15] Increased circulating hepcidin, resulting from inflammatory stimulation of hepcidin production and decreased hepcidin clearance, restricts the release of iron into the plasma, causing hypoferraemia. IV iron administration loads macrophages with iron, thereby stimulating ferroportin synthesis in macrophages. Increased ferroportin facilitates the export of iron trapped in macrophages out of the cell for erythropoiesis, overcoming the effect of high levels of hepcidin in the plasma and making more iron available for stimulated erythropoiesis.

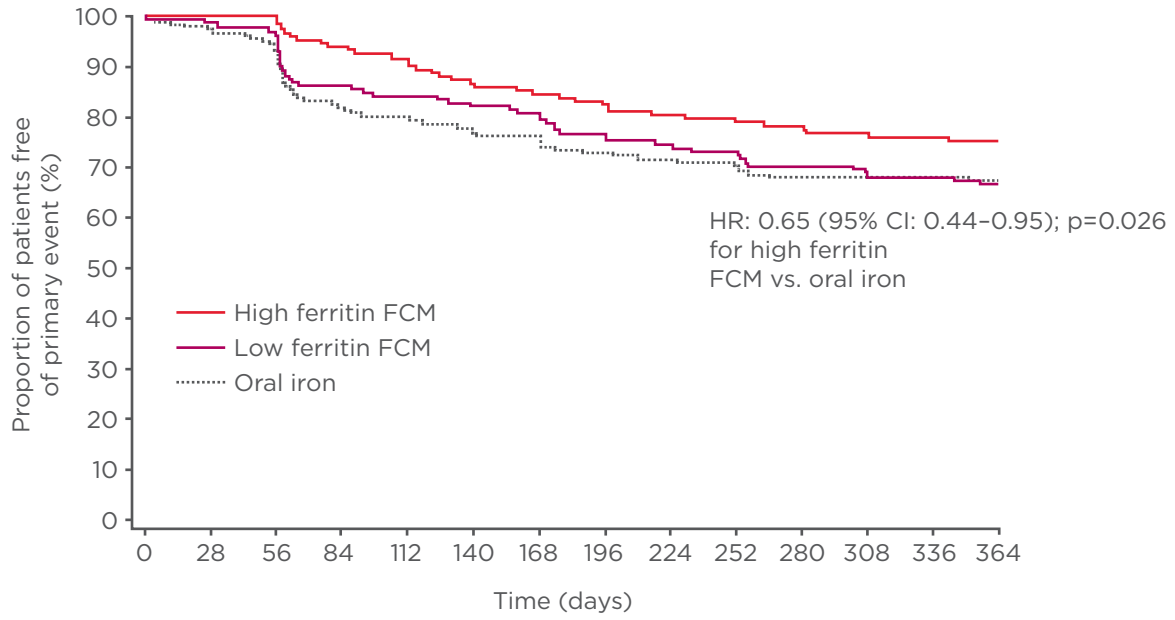
Diagnosing and Treating Iron Deficiency/Iron-Deficiency Anaemia: Meeting Your Patient's Needs

Professor Jolanta Małyszko

The typical patient undergoing haemodialysis has impaired EPO production and EPO receptor function, impaired iron absorption, iron loss during their haemodialysis sessions, inflammation,

and increased iron utilisation (following the administration of EPO-stimulating agents), all of which can lead to iron deficiency and anaemia. Although patients with ND-CKD appear to be less anaemic, they are still iron deficient as a result of impaired iron absorption and repeated venepuncture, with approximately 60% of patients with ND-CKD who start on dialysis being deficient in iron.[16] Assessment of iron deficiency prior to iron therapy is important; this is usually done by measuring the levels of serum ferritin, serum iron, TSAT, and total iron binding capacity; and assessing reticulocyte haemoglobin content, measuring occult blood in stools, determining red blood cell indices, and measuring levels of haemoglobin.[17] Iron stores should be evaluated and non-renal causes of anaemia should be excluded from this assessment.[17] Often, defining iron deficiency using serum ferritin levels and TSAT is difficult in the CKD population, as these biochemical markers can often be affected by acute-phase reactions, particularly those seen in inflammatory disease states such as diabetes and cardiovascular disease, diseases that commonly occur in this population. The advantages of assessment of iron stores using serum ferritin levels as a measure include: high specificity of low levels of this haematological parameter being indicative of iron deficiency;[18] its correlation with body iron stores in healthy individuals;[19] and its ease-of-use, moderate cost, and wide availability. However, normal or high serum ferritin does not exclude functional iron deficiency[20] and there are also observed gender differences in this measurement.[21] In contrast, TSAT is a more reliable measure of iron deficiency than serum ferritin as it is more sensitive[18] and the absence (or near-absence) of sustainable iron in the bone marrow correlates with TSAT <20%.[21] It must be noted that, in patients undergoing dialysis, there is 17–70% diurnal variation in TSAT levels[20,21] and levels can be affected by inflammation, malnutrition, and chronic disease, interfering with its reliability as a measurement of iron deficiency.[21]

Clinical guidelines recommend treating iron-deficiency anaemia with oral or IV iron before initiating other anaemia management,[22,23] as optimal red blood cell production requires iron for haemoglobin synthesis.[24,25] Iron losses in patients with CKD undergoing haemodialysis can be attributed to repeated laboratory tests, accidental losses during haemodialysis and other bleeding events, blood retention in the artificial kidney and tubing, and normal iron losses; these incremental losses can result in the loss of up to 3,000 mg of iron per year.[18]



High serum ferritin FCM	153	153	150	139	134	126	123	118	114	112	110	107	106	81
Low serum ferritin FCM	152	150	145	129	124	121	117	109	106	103	96	93	93	75
Oral iron	308	299	278	241	229	219	212	203	198	194	185	185	184	151

Figure 2: Primary endpoint results of the FIND-CKD study.[30]
FCM: ferric carboxymaltose; HR: hazard ratio; CI: confidence interval.

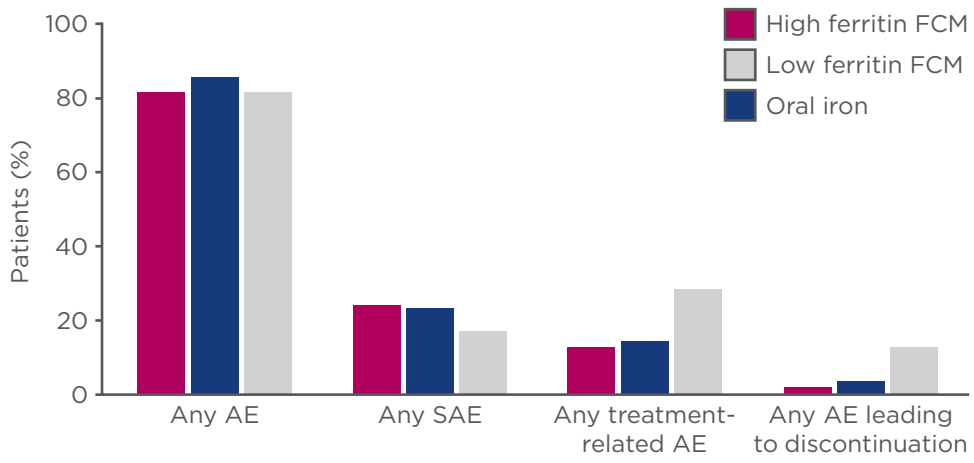


Figure 3: Safety results across treatment groups in the FIND-CKD study.[30]
FCM: ferric carboxymaltose; AE: adverse events; SAE: serious adverse events.

In patients with CKD on haemodialysis, the administration of parenteral iron is routinely employed due to the loss of blood associated with haemodialysis, the need for adequate levels of iron in response to EPO administration, and because patients are often unable to respond to oral iron. In ND-CKD patients, oral or IV iron therapy is initiated depending on the severity of anaemia. ND-CKD patients with severe anaemia may have gastrointestinal intolerance for oral iron therapy and their iron deficiency is unlikely to be corrected within 3 months of receiving oral iron administration. As in patients on haemodialysis, those receiving EPO-stimulating agents are also recommended for IV iron therapy.[22]

Oral iron treatment offers several advantages: it is widely used, inexpensive, and easily administered,[20,26] with no requirement for outpatient visits.[20] However, adherence can be a problem, the underlying blood loss pathology is often not resolved,[27] and iron absorption can be inhibited due to other medications or diet.[28] Oral iron can also lead to frequent gastrointestinal side effects, such as nausea, constipation, and diarrhoea.[28]

IV iron treatment has shown benefits in patients with ND-CKD. The FIND-CKD study was the largest and one of the longest (56-week) randomised studies comparing IV and oral iron in patients with ND-CKD.[29] The study recruited >600 patients with a haemoglobin level of 9–11 g/dL, serum ferritin <100 µg/L, or serum ferritin <200 µg/L + TSAT <20%. The three treatment groups were IV ferric carboxymaltose (FCM) (200 and 1,000 mg) and oral ferrous sulphate (200 mg iron/day), with a primary endpoint of time to initiation of an alternative treatment for anaemia or occurrence of a haemoglobin trigger (specified as two consecutive haemoglobin values <10 g/dL on or after Week 8, without an increase of ≥0.5 g/dL between consecutive values). Secondary endpoints included the percentage of patients with an increase of haemoglobin ≥1 g/dL, and a change in haematological and iron indices. Results showed that 76% of patients with ND-CKD maintained a haemoglobin level ≥10 g/dL or did not require further anaemia treatment when treated with FCM targeting high serum ferritin levels (Figure 2).

FCM targeting of a higher ferritin level also achieved a faster and greater increase in haemoglobin levels versus oral iron. High ferritin FCM also resulted in the desired serum ferritin targets being achieved, and TSAT levels were maintained within guideline recommendations versus oral iron for all time points ($p<0.001$).[29] There were no changes in adverse events between the FCM groups targeting high and low serum ferritin levels, but there were higher rates of adverse events leading to treatment discontinuation in the oral iron group (Figure 3). Importantly, there was no sign of renal toxicity in the FCM group targeting ferritin levels of 400–600 µg/L.

IV iron therapy is well established in patients on haemodialysis, however the benefits of IV iron therapy beyond red blood cell management is still a point of discussion in ND-CKD patients, although the FIND-CKD, a 1-year study with FCM, suggests a faster and greater haemoglobin response with IV iron compared with oral iron in ND-CKD patients.[29] Further research to establish benefits and risks of IV iron therapy is desired.[31]

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