Diagnostic tests and therapeutic targets for anemia of CKD

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DISCLOSURES

I am an employee of the Radboud University Medical Center that serves the medical, scientific and commercial community with hepcidin reference material and hepcidin and toxic iron measurements at a fee-for-service basis (www.hepcidinanalysis.com)

I participate in the clinical and scientific advisory board of Silence therapeutics, that develops hepcidin targeting compounds
Biomarkers of iron metabolism

- Ferritin
- Transferrin
- Iron/NTBI
- Hepcidin
- sTfR
- Retis, Erys
Absolute iron deficiency

Iron stores
- spleen
- liver

Bone marrow erythropoiesis

Circulation

- ferritin
- hepcidin
- iron
- transferrin
- ferri-transferrin
- sTfR

reti’s or ery’s

KDIGO
Primary overload syndromes, hereditary hemochromatosis

- Iron stores
- Bone marrow erythropoiesis
- Circulation

**Iron stores**
- Ferritin
- Hepcidin
- Iron/NTBI
- Transferrin
- Ferrit-transferrin
- sTfR
- Retis or erys (erythrocytes)

**Bone marrow erythropoiesis**

**Circulation**
Functional iron deficiency (iron distribution disorder)

Iron stores

Bone marrow erythropoiesis

Circulation

Iron-restricted erythropoiesis: insufficient iron mobilization from the (otherwise adequate) body iron stores to meet iron demands of erythroid precursors.
Elevated hepcidin $\rightarrow$ iron distribution/mobilisation disorder
Treatment with ESA $\rightarrow$ high iron demand

Functional iron deficiency
**Ferritin**

- Reflects overall storage iron (but is chiefly derived from macrophages)
- Reference values vary and depend on age, gender (and race), and are not always useful as cut-off
- Robust data on clinical decision limits/diagnostic accuracy/thresholds are lacking
- Levels are increased in patients with liver diseases, metabolic syndrome, inflammation, infection
- Levels are decreased in absolute ID;
- Overall, iron deficiency < 12-30 (specific); iron replete > 100; iron overload > 200-300 µg/l
- In CKD, proposed levels:
  - absolute ID in non-HD < 100 µg/l and HD < 200 µg/l
  - functional ID vary between 100-1200 µg/l
- In CKD: inflammation and elevated hepcidin levels → iron distribution to RES
  - (low TSAT and) relatively high ferritin for body iron levels
  - long term safety of RES iron loading in CKD is unclear
- MRI liver cannot distinguish between parenchymal and RES iron overload.
- Algorithemes to correct ferritin for inflammatory markers are not universally applicable (vary with type of inflammation, stage of disease)

Blackmore, 2008; Ferraro, 2018; Harris, 2007; KDIGO, 2012; Thomas, 2013; Thurnham, 2010; Suchdev, 2017; Daru, 2017
TRANSFERRIN SATURATION (TSAT %)

- Reflects circulating iron levels
- Comprises 2 measurements: 1. iron and 2. TIBC (=UIBC + Iron) or transferrin
- Calculation: TSAT (%) = iron/ TIBC x 100 %; TIBC (µmol/L) = transferrin (g/L) x 25.2
- In patients with inflammation/CKD, iron is more decreased than transferrin → decrease in TSAT → less iron available for erythropoiesis
- TSAT < 20%: absolute or functional iron deficiency; some guidelines for CKD < 25-30%
- TSAT > ≈80%, formation of toxic iron forms (non transferrin bound iron, NTBI)
- In patients with hyperferritinemia,
  - high TSAT is associated with primarily parenchymal iron overload
  - low TSAT is associated with primarily RES iron overload
- In patients with non-HD CKD, low TSAT is associated with higher mortality

Thomas DW, BJH 2013; KDIGO, 2012l Kovesdy CP, CJASN 2009; Eisinga M, BMC Nephrology 2018
HEPCIDIN in CKD results from the relative strengths of opposing stimuli

Swinkels & Wetzels, NDT 2008; Yamada, Kidney Int 2009
Hepcidin is increased in most patients with CKD

In majority of studies controls and patients are not matched for age, gender and iron supplementation (ferritin)
## Hepcidin and CKD

<table>
<thead>
<tr>
<th>Parameter/outcome</th>
<th>study population</th>
<th>association with hepc.</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>elevated ferritin</td>
<td>HD/non-HD</td>
<td>+++</td>
<td>major determinant</td>
</tr>
<tr>
<td>low GFR</td>
<td>non-HD</td>
<td>+</td>
<td>inconsistent</td>
</tr>
<tr>
<td>CRP and IL-6</td>
<td>HD/non-HD</td>
<td>++</td>
<td>in most studies</td>
</tr>
<tr>
<td>ESA resistance or response</td>
<td>HD</td>
<td>+/-</td>
<td>inconsistent</td>
</tr>
<tr>
<td>effect of iron supplementation on Hb</td>
<td>HD</td>
<td></td>
<td>small study, no control group</td>
</tr>
<tr>
<td>type of dialyzer</td>
<td>HD</td>
<td>+/-</td>
<td>inconsistent</td>
</tr>
<tr>
<td>renal anemia</td>
<td>non-HD</td>
<td>+</td>
<td>prediction</td>
</tr>
<tr>
<td>atherosclerosis</td>
<td>HD</td>
<td>+</td>
<td>CV events/arterial stifness</td>
</tr>
</tbody>
</table>

High within subject variation in hepcidin in time

**Conclusion**

Hepcidin alone is:
1. not an anemia management tool
2. A biomarker for cardiovascular disease?

Hemoglobin

- Overall, the exact Hb threshold below which a health outcome is detrimental is undefined.
- In CKD, Hb level at which ESA treatment should be initiated to increase quality of life is unclear.
- In CKD, Hb target for ESA treatment is 10-12 g/L, based on optimal ratio benefit (quality of life)/risk (stroke and thromboembolic events).
- Whether Hb targets in CKD should depend on age, gender, ethnicity, genetic factors, altitude, and hypoxia (smoking) is unclear.
- Hb not only reflects body iron status; in CKD also other factors contribute to anemia: inflammation, reduced red blood cell survival and genetic factors.

NOTE: also ID (without anemia) may cause symptoms.
RED BLOOD CELL MARKERS

RetHb content
• Reticulocyte Hb content is Hb content (MCH) of reticulocytes and available on various automated hematology platforms
• Early marker for iron restricted erythropoiesis due to absolute or functional iron deficiency (before development of anemia)
• Monitoring response to therapy:
  • a guidance for diagnosing functional ID and optimizing iron therapy in patients receiving ESA for end stage renal failure
  • an early marker of erythropoietic response to iron supplementation
• Absence of clinical decision limits

% hypochromic cells:
• Time averaged marker of iron restricted erythropoiesis
• Diagnosing functional ID in patients receiving ESA
• Sensitive to pre-analytical bias (time to analysis)
• Absence of clinical decision limits.

**sTfR**

- Increases when iron availability does not meet erythropoietic needs
- Less suitable as a marker of functional ID in CKD since:
  - CKD patients often have erythroid hypoplasia (that masks ID-induced increases)
  - in CKD patients receiving ESA, sTfR reflects more erythroid response to ESA than to (functional) ID

**ERYTHROFERRONE**

- Produced by erythroblast in response to EPO; inhibits hepcidin production
- Elevated in HD-CKD with dose response to ESA treatment
- No clear relation with hepcidin in CKD
- Associated with mortality and CV events in non-HD and HD CKD patients, mechanism needs elucidation

Eschbach, 1992; Fernandez-rodriguez, 1999; Ahluwalia, 1997; Beguin 1993; Wish, 2006; Huebers, 1990; Spoto, 2019; Honda, 2016; Hanudel, 2018
Several iron and RBC biomarkers are not standardized

**Standardized:** automated Hb

**Moderately standardized:** ferritin and transferrin/TIBC (Blackmore 2018)

**Non standardized:** sTfR (Thorpe, 2010; Pfeiffer, 2017), erythroferrone, RetHb, % hypochromic cells, hepcidin*

*Hepcidin standards have recently been developed allowing standardisation (van der Vorm, 2016; Diepeveen, 2019)
Compounds that interfere with iron metabolism in CKD and affect iron biomarkers

HEPCIDIN ANTAGONISTS
• Multiple strategies that counter the effect of hepcidin in iron-restrictive disorders (such as CKD) have been described
• Phase 1 and 2 of some of these compounds have been completed.
• Some programs have been stopped after phase 1/2
• Overall, these compounds show increase in TSAT and decrease in hepcidin levels in healthy volunteers, and patients with inflammatory diseases and CKD; clear effects on Hb, RetHb in CKD patients have yet to be shown.

HIF STABILIZERS
• Decrease hepcidin levels (EPO dependent and independent)
• Increase in intestinal iron absorption
• Increase use of iron via hepcidin dependent and independent mechanism

Schwoebel, 2013; Boyce, 2016; van Eijk 2014, Hohlbaum, 2018, Galli, 2018; Sheetz, 2019; Barrington, 2016 (abstract); Petzer, 2018 (review); Renders, 2019; Anderson 2013; Koury, 2015 (review)
Combined iron and RBC biomarkers provide insight in iron dyshomeostasis in CKD, and may contribute to treatment selection

**Absolute Iron Deficiency (Anemia)**
- Low body iron stores
- Inadequate total iron available,
- Low ferritin
- Low transferrin saturation%,
- Low RetHb, High sTfR, Low hepcidin
- Iron supplementation

**Functional Iron Deficiency**
- Normal body iron stores
- Inadequate mobilisation of iron
- Normal/elevated ferritin, Low transferrin saturation%,
- Low RetHb, Low- High sTfR, elevated hepcidin
- Elevated inflammatory markers
  - ESA, anti-hepcidin,
  - HIF-stabilizer
- (iron supplementation)

**Anemia of chronic disease**
- Erythroid suppression
- Reduced red cell survival
- Rel. EPO def.

- Absolute ID and functional ID partly overlap
- Absolute ID and anemia of chronic disease partly overlap
- Functional Iron Deficiency and Anemia of Chronic Disease largely overlap

Thanks to SR Pasrisha
TAKE HOME

➢ Several conventional and innovative iron and RBC biomarkers
  - derived from stores, circulation and bone marrow

➢ Suitability biomarkers to define treatment strategies
  - individual biomarker: low
  - combination biomarkers (in clinical context): relatively high

➢ High ferritin
  + elevated TSAT → primarily parenchymal loading= rel. toxic
  + normal/low TSAT→ primarily RES loading=rel. safe (long term effects unknown)

➢ Clinical interpretation of biomarker results
  - often method dependent since assays are not standardized
  - low evidence for clinical decision limits: largely expert opinion base
FUTURE DIRECTIONS AND RESEARCH NEEDS

• Standardization of iron and RBC biomarkers

• Elucidating clinical decision limits relevant for iron adequacy in CKD

• Need for functional markers of iron deficiency beyond Hb

• Identification of genetic loci that are associated with iron metabolism in CKD and treatment outcomes → personalized treatment strategies