The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/138156

Please be advised that this information was generated on 2020-06-15 and may be subject to change.
Hematologic parameters predicting a response to oral iron therapy in chronic inflammation

In anemic patients with chronic inflammatory conditions, the assessment of iron status and the selection of those who may benefit from iron therapy frequently presents diagnostic challenges.\textsuperscript{1-3} Traditional biochemical iron parameters, such as ferritin, serum iron and transferrin, are affected by inflammation making them less suitable indicators.\textsuperscript{4}

Recently, iron parameters have become available that provide direct information on iron availability for erythropoiesis: the central iron regulatory hormone hepcidin\textsuperscript{5-7} and the hemoglobin content of reticulocytes and erythrocytes (Ret-He and RBC-He).\textsuperscript{8-11} We hypothesized that changes in these functional iron parameters shortly after start of iron supplements may predict an increase in hemoglobin in a longer course of iron supplements in patients with chronic inflammation.

Forty adult patients diagnosed with a chronic rheumatic disease and anemia were selected during routine visits in 2012 at the rheumatology outpatient clinic of the Radboud University Medical Center, Nijmegen, the Netherlands. Anemia was defined as a hemoglobin concentration below 12.1 g/dL for males and below 11.3 g/dL for female, respectively. Exclusion criteria were signs of hemolysis (increased levels of lactate dehydrogenase), end stage renal disease, liver disease (two times the upper limit of the reference value of alanine aminotransaminase (ALT)), known malignancies, vitamin B12 or folic acid deficiency, use of iron supplements, erythropoietin or medications with potential drug interactions with the iron supplements and a blood transfusion in the previous two months. Patients’ characteristics, including age, sex, medication and the type of inflammatory condition, were recorded at baseline (Table 1).

Rheumatoid arthritis was the most common diagnosis (58%) and disease-modifying drugs (60%) and non-steroidal anti-inflammatory drugs (53%) were frequently used. Ethical clearance was granted by the Radboud University Medical Center, and written informed consent was obtained from all participants.

Ferrofumarate 200 mg BD (130 mg of elemental iron per day) was prescribed during six weeks. Blood samples were collected at baseline, after one, two and six weeks. Responders to iron therapy were defined as patients with an increase in hemoglobin of at least 0.81 g/dL (0.5 mmol/L) after six weeks of oral iron supplementation. The hemoglobin content parameters were measured on a Sysmex XE5000 analyser (Sysmex Corporation). Serum hepcidin-25 measurements were performed using a combination of weak cation exchange chromatography and time-of-flight mass spectrometry as described previously.\textsuperscript{12}

The other iron parameters were determined as previously described.\textsuperscript{13}

At week 6, 2 patients had withdrawn from the study: one at personal request and one because of pneumonia. Sixteen of 38 participants who completed the study were responders. Responders had significantly lower base-line values of ferritin, hepcidin, Ret-He and RBC-He and transferrin saturation (TS), and higher total iron binding capacity (TIBC), soluble transferrin receptor (sTfR) and its ratio to log ferritin (sTfR-index) levels in comparison to non-responders (Figure 1). Levels of CRP were similar between the groups. At one week of iron therapy, responders had a significantly larger increase in Ret-He level (0.28 ± 0.26 fmol) compared to the non-responders (0.18 ±0.04 fmol) (P<0.000) and a larger change in reticulocyte numbers (Figure 1). There was no increase in Ret-He between weeks 1 and 2. In contrast, there were no significant differences in the change in hepcidin levels at either time point. Moreover, responders had significantly larger increases in serum iron and TS values at one week with a concurrent significant decline in sTfR-index levels after two weeks of iron therapy.

Regression analyses were applied to predict the change in hemoglobin and the outcome of being a responder after six weeks of iron therapy. The strongest association with the hemoglobin response at six weeks was found for baseline RBC-He, ferritin, Ret-He and hepcidin levels, and the increase after one week in values of TS, serum iron, Ret-He, reticulocytes and ferritin, both in descending order (Table 2).

The diagnostic performance of parameters was subsequently assessed using Receiver Operating Characteristic (ROC) analyses. The change in values of serum iron, TS, Ret-He and reticulocytes at one week had the highest areas under the curve (AUC) (Table 2). At one week, an increase in serum iron and Ret-He of more than 4 mmol/L and 0.11 fmol, respectively, had a sensitivity of 80% and 81%, and specificity of 91% and 86%, respectively. At baseline, values of ferritin, hepcidin and the sTfR-index had the highest AUC values, but the change in these parameters at week 1 was not a useful predictor of the response to iron therapy. The combination of the change in values of Ret-He and TS at one week provided the highest discriminatory power (AUC 0.906, SE 0.054; P<0.000).

Overall, the increase after one week of oral iron therapy in Ret-He, TS, serum iron level and the reticulocyte count best predicted an increase in hemoglobin level of at least

| Table 1. Base-line characteristics of study population. |
|----------------|----------------|
| Variable                  | Total (n=40) |
| Female, n. (%)            | 26 (65)      |
| Age, mean (SD), y         | 62 (15)      |
| Condition, n. (%)         |               |
| Rheumatoid arthritis      | 23 (57.5)    |
| Spondyloarthropathy       | 2 (5)        |
| PMR                       | 3 (7.5)      |
| Arthritis ns              | 6 (15)       |
| Other auto-immune disease | 6 (15)       |
| Drugs*, n. (%)            |               |
| NSAID                     | 21 (52.5)    |
| Glucocorticosteroids      | 20 (50)      |
| DMARD                     | 24 (60)      |
| Biological                | 7 (17.5)     |
| Combination of 2          | 16 (40)      |
| Combination of 3 or more  | 9 (22.5)     |
| Hemoglobin, mean (SD), g/dL|            |
| Male                      | 11.8 (0.9)   |
| Female                    | 11.3 (0.7)   |
| CRP, median (IQR), mg/L   | 9.5 (4.3-20.3)|
| Creatinine, mean (SD), μmol/L | 75.9 (28.9) |
| ALT, mean (SD), U/L       | 29.2 (15.2)  |
| Folic acid, mean (SD), nmol/L | 22.7 (13.8) |
| Vitamin B12, mean (SD), pmol/L | 350.7 (164.0) |

*Drugs related to inflammatory condition. SD: standard deviation; PMR: polymyalgia rheumatica; ns: non-specified; NSAID: non-steroidal anti-inflammatory drugs; DMARD: disease modifying drugs; CRP: C-reactive protein; IQR: interquartile range; ALT: alanine aminotransferase.
LETTERS TO THE EDITOR

p-values of paired samples t-test or Wilcoxon Signed Rank test comparisons between baseline and week one or week two values (when applicable): Ret-He 2.00-2.41 fmol/L, reticulocytes 20-80 x 10^9/L, hepcidin < 14.7 (males), < 12.3 nM (premenopausal females), and non-responders (gray). *represent significant P-values of t-test comparisons or Mann Whitney tests between responders and non-responders.

Ferritin, µg/L

TS, %

25.4 % ; -1.4,

0.824± 0.088; P 0.001

0.783± 0.080; P 0.004

0.771± 0.087; P 0.001

0.783± 0.080; P 0.004

0.808± 0.075; P 0.001

0.765± 0.010; P 0.002

0.774± 0.079; P 0.004

0.783± 0.079; P 0.004

0.828± 0.067; P 0.001

0.762± 0.081; P 0.007

0.727± 0.090; P 0.002

0.716± 0.089; P 0.03

RBC-He: erythrocyte-hemoglobin content; TIBC: total iron binding capacity; TS: transferrin saturation; sTR: soluble transferrin receptor; sTRIndex: sTR/ log ferritin; ROC: receiver operator curve; SI conversion factors: To convert hemoglobin to grams per liter, multiply by 10.0; to convert Ret-He, RBC-He to pg, multiply by 16.1.

Table 2. Prediction of the response to six weeks of iron therapy.

Linear regression

At baseline (n=38)

Change at 1 week (n=38)

Change at 2 weeks (n=37)

Reticulocytes, 10^9/L

n.s.

31.1 % 2.94, P<0.001

23.7 % 2.15, P 0.001

Ret-He, fmol

40.5 % -6.2, P<0.001

24.9 % 4.4, P 0.001

19.8 % 4.1, P 0.003

RBC-He, fmol*

52.8 % -7.9, P<0.001

22.5 % 22.9, P 0.005

41.5 % 21.8, P<0.001

Hepcidin, nmol/L

35.4 % -56, P<0.001

ns

ns

Iron, µmol/L

16.8 % -13, P 0.006

47.5 % 1.04, P<0.001

21.9 % 0.77, P 0.002

TIBC, µmol/L

22.8 % 4.5, P 0.008

ns

ns

TS, %

25.4 % -1.4, P 0.001

48.9 % 1.04, P<0.001

23.7 % 0.76, P 0.001

Ferritin, µg/L

41.4 % -80, P<0.001

17.8 % 0.93, P 0.006

20.1 % 1.0, P 0.003

sTR, mg/L

24.1 % 1.8, P 0.001

ns

ns

sTR-index, mg/µg

43.9 % 15, P 0.001

16.0 % -2.6, P 0.009

23.7 % -3.2, P 0.001

ROC analyses

Reticulocytes, 10^9/L

ns

$0.807± 0.073; P 0.001

0.783± 0.090; P 0.004

Ret-He, fmol

0.720± 0.088; P 0.02

0.824± 0.088; P 0.001

0.783± 0.080; P 0.004

RBC-He, fmol*

0.771± 0.087; P 0.001

ns

0.765± 0.010; P 0.002

Hepcidin, nmol/L

0.808± 0.075; P 0.001

ns

ns

Iron, µmol/L

ns

0.894± 0.058; P 0.000

0.786± 0.080; P 0.003

TIBC, µmol/L

0.774± 0.079; P 0.004

ns

ns

TS, %

ns

0.889± 0.058; P 0.000

0.783± 0.079; P 0.004

Ferritin, µg/L

0.828± 0.067; P 0.001

ns

0.762± 0.081; P 0.007

sTR, mg/L

0.727± 0.090; P 0.002

ns

ns

sTR-index, mg/µg

0.801± 0.079; P 0.002

ns

0.716± 0.089; P 0.03

Figure 1. Box and whisker plots of iron status parameters at baseline and after one and two weeks of iron supplementation for responders (white) and non-responders (gray). *represent significant P-values of t-test comparisons or Mann Whitney tests between responders and non-responders.
0.81 g/dL at week 6 of iron therapy. Base-line values of ferritin, hepcidin, sTfR-index and RBC-He also predicted the hematopoietic response, but to a lesser degree.

There are still no established diagnostic criteria to identify absolute iron deficiency in patients with inflammation. Our study design is unique in that we evaluated the predictive value of iron status indicators in response to oral iron therapy in patients with rheumatic diseases. The value of Ret-He as a screening tool for ID has been recognized before and our current findings support its role as a marker for iron responsiveness. This may be of particular diagnostic value when results of conventional iron status parameters are inconclusive. The advantage of reticulocyte count and hemoglobin content parameters is that their results can be obtained quickly and extra blood tests are not required. However, before these hemoglobin content parameters can be widely implemented, additional research into the various patients’ groups and clinician education and training is required.

Our study was designed with minimal inclusion criteria in order to maximize the generalizability of our results. Potential limitations are the self-reported drug compliance: i) the possible interaction of iron absorption with frequently used proton pump inhibitors (specific intake instructions were given); ii) the relatively modest degree of inflammation in our study population; and, finally, iii) the duration of iron treatment in our study may not have been sufficient to replete iron stores in all patients.

In conclusion, in anemic patients with a chronic rheumatologic disease, the increase in hemoglobin concentration to six weeks of iron supplementation was best predicted by the change in Ret-He, TS and serum iron levels and the reticulocyte count after one week of oral iron therapy. Prospective analysis of these parameters is especially useful when initial values of iron status indicators, such as a normal or elevated serum ferritin level, cannot be used to reliably exclude iron deficiency.

Susanne van Santen, 1,2 Quirijn de Mast, 1 Janine D. Oosting, 3 Annelies van Ede, 4 Dorine W. Swinkels, 1 and André J.A.M. van der Ven1

1Department of General Internal Medicine; 2Department of Laboratory Medicine, Laboratory of Genetic, Endocrine and Metabolic diseases; 3Department of Laboratory Medicine, Laboratory of Clinical Chemistry; and 4Department of Rheumatology, Radboud University Medical Center, Nijmegen, the Netherlands

Correspondence: Andre.vanderVen@radboudumc.nl
doi:10.3324/haematol.2014.106799

Key words: hematologic parameters, oral iron therapy, chronic inflammation, response.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References