

## 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.<sup>1,2</sup> Traditional biochemical iron parameters, such as ferritin, serum iron and transferrin, are affected by inflammation making them less suitable indicators.<sup>3</sup>

Recently, iron parameters have become available that provide direct information on iron availability for erythropoiesis: the central iron regulatory hormone hepcidin<sup>4,7</sup> and the hemoglobin content of reticulocytes and erythrocytes (Ret-He and RBC-He).<sup>8-11</sup> 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 analyzer (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.<sup>12</sup> The other iron parameters were determined as previously described.<sup>7</sup>

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 ( $0.28 \pm 0.26$  fmol) compared to the non-responders ( $0.18 \pm 0.04$  fmol)

**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)
Spondylarthropathy	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), $\mu$ mol/L	75.9 (20.9)
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)

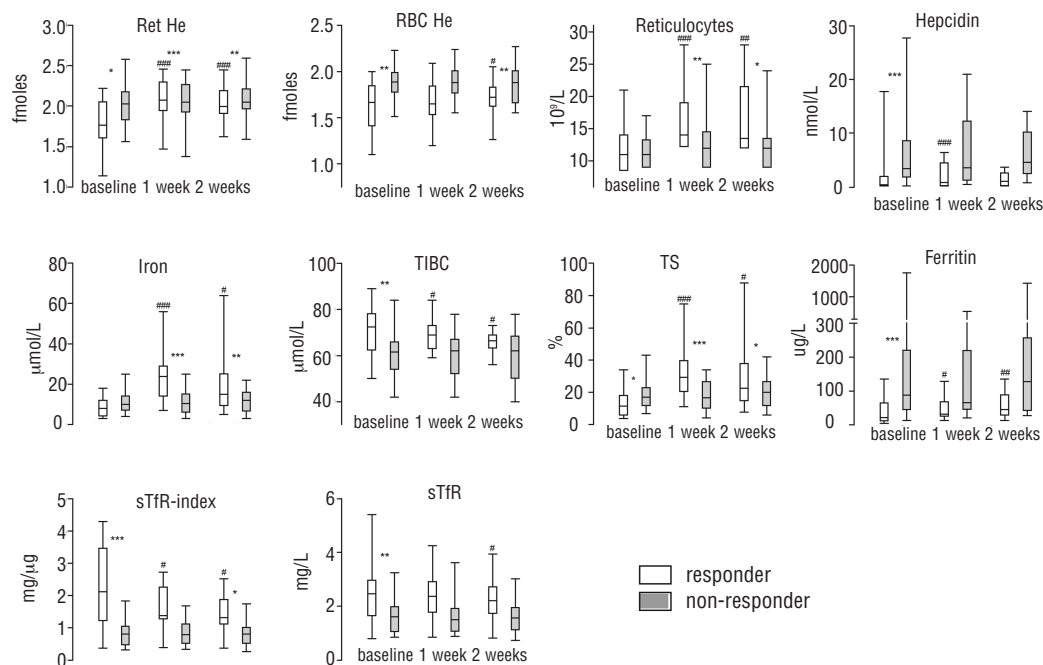
\*Drugs related to inflammatory condition. SD: standard deviation; PMR: polymyalgia rheumatica; ns: non specified; NSAID: non-steroidal anti-inflammatory drugs; DMARD: disease modifying drugs; CRP: C-reactive protein; IQR: interquartile range; ALT: alanine aminotransferase.

( $P < 0.000$ ) 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



**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. #represent significant *P*-values of paired samples t-test or Wilcoxon Signed Rank test comparisons between baseline and week one or week two of responders and non-responders. *P*-values xxx  $\leq 0.001$ , xx  $\leq 0.01$ , x  $< 0.05$ . For abbreviations and SI conversion factors see Table 2. Reference values (when applicable): Ret-He 2.00-2.41 fmoles, reticulocytes 20-80  $10^9/L$ , hepcidin  $< 14.7$  (males),  $< 12.3$  nM (premenopausal females),  $< 15.6$  nM (postmenopausal women), iron 10-30  $\mu\text{mol/L}$ , TIBC 45-80  $\mu\text{mol/L}$ , ferritin 15-180  $\mu\text{g/L}$  (males), 6-80  $\mu\text{g/L}$  (premenopausal females), 15-280  $\mu\text{g/L}$  (postmenopausal women), sTfR 0.76-1.76 mg/L.

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

Linear regression <sup>†</sup>	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, $\mu\text{mol/L}$	16.8 %; -1.3, $P 0.006$	47.5 %; 1.04, $P < 0.001$	21.9 %; 0.77, $P 0.002$
TIBC, $\mu\text{mol/L}$	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, $\mu\text{g/L}$	41.4 %; -80, $P < 0.001$	17.8 %; 0.93, $P 0.006$	20.1 %; 1.0, $P 0.003$
sTfR, mg/L	24.1 %; 1.8, $P 0.001$	ns	ns
sTfR-index, mg/ $\mu\text{g}$	43.9 %; 1.5, $P < 0.001$	16.0 %; -2.6, $P 0.009$	23.7 %; -3.2, $P 0.001$
ROC analyses <sup>‡</sup>			
Reticulocytes, $10^9/L$	ns	$\$0.807 \pm 0.075$ ; $P 0.001$	$0.783 \pm 0.090$ ; $P 0.004$
Ret-He, fmol	$0.720 \pm 0.088$ ; $P 0.02$	$0.824 \pm 0.080$ ; $P 0.001$	$0.783 \pm 0.080$ ; $P 0.004$
RBC-He, fmol*	$0.771 \pm 0.087$ ; $P 0.01$	ns	$0.765 \pm 0.101$ ; $P 0.02$
Hepcidin, nmol/l	$0.808 \pm 0.075$ ; $P 0.001$	ns	ns
Iron, $\mu\text{mol/L}$	ns	$0.894 \pm 0.058$ ; $P 0.000$	$0.786 \pm 0.080$ ; $P 0.003$
TIBC, $\mu\text{mol/L}$	$0.774 \pm 0.079$ ; $P 0.004$	ns	ns
TS, %	ns	$0.889 \pm 0.058$ ; $P 0.000$	$0.783 \pm 0.079$ ; $P 0.004$
Ferritin, $\mu\text{g/L}$	$0.828 \pm 0.067$ ; $P 0.001$	ns	$0.762 \pm 0.081$ ; $P 0.007$
sTfR, mg/L	$0.727 \pm 0.090$ ; $P 0.02$	ns	ns
sTfR-index, mg/ $\mu\text{g}$	$0.801 \pm 0.079$ ; $P 0.002$	ns	$0.716 \pm 0.089$ ; $P 0.03$

Table shows the (i) linear regression model to predict the change in hemoglobin at week 6 and (ii) ROC analyses to assess the chance of being responder of base-line parameters and their relative changes after one or two weeks of iron therapy. <sup>†</sup>Depicted are the adjusted squared *R* ( $\times 100\%$ ), the unstandardized *B*-coefficient and *P*-value, calculated on log-transformed variables. The *B*-coefficient expresses the (relative) change in hemoglobin concentration after six weeks iron therapy that is associated with a 1-unit change in the independent variable. <sup>‡</sup>Depicted are: the area under the curve, standard error and corresponding *P*-value. \**n*: 32, *n*: 36 and *n*: 34, respectively. Ret-He: reticulocyte-hemoglobin content; ns: non-significant; RBC-He: erythrocyte-hemoglobin content; TIBC: total iron binding capacity; TS: transferrin saturation; sTfR: soluble transferrin receptor; sTfR-index: sTfR/ 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.

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<sup>7,13</sup> 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.<sup>14</sup>

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.

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