The role of hepcidin and an oral iron absorption test in identifying the root cause of iron-restricted anemia

Loveikyte, R.; Berg, Y. van den; Jong, A. de; Vlasveld, L.T.

Citation
Loveikyte, R., Berg, Y. van den, Jong, A. de, & Vlasveld, L. T. (2023). The role of hepcidin and an oral iron absorption test in identifying the root cause of iron-restricted anemia. *Acta Haematologica*. doi:10.1159/000535275

Version:	Publisher's Version
License:	Creative Commons CC BY 4.0 license
Downloaded from:	https://hdl.handle.net/1887/3731725

Note: To cite this publication please use the final published version (if applicable).

Acta Haematologica

Original Paper

Acta Haematol
DOI: 10.1159/000535275

Received: July 7, 2023
Accepted: November 13, 2023
Published online: November 16, 2023

The Role of Hepcidin and an Oral Iron Absorption Test in Identifying the Root Cause of Iron-Restricted Anemia (Enter-Iron)

Roberta Loveikyte[a, b] Yascha van den Berg[c]
Andrea Elisabeth van der Meulen-de Jong[a] Lodewijk Thomas Vlasveld[c]

[a]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, The Netherlands; [b]Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, The Netherlands; [c]Department of Internal Medicine, Haaglanden Medical Center, The Hague, The Netherlands

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

Keywords
Anemia · Hepcidin · Oral iron absorption · Iron deficiency

Abstract

Introduction: Traditional iron parameters often fail to distinguish the cause of iron-restricted anemia in patients without an obvious underlying cause. We evaluated whether an oral iron absorption test (OIAT) and hepcidin measurement could be useful diagnostic tests in these patients. ***Methods:*** We retrospectively analyzed data extracted from medical records of all patients who underwent an OIAT and hepcidin measurement, noting subsequent clinical diagnosis. Δ Iron >15 μmol/L during the OIAT and a hepcidin level below the median (or suppressed ≤0.5 nM) were considered appropriate. ***Results:*** Thirty-nine adult patients were included in the study. Sixteen patients with adequate OIAT had suppressed hepcidin levels indicative of classical iron-deficiency anemia (IDA); 59% of patients had abnormal OIAT. In this group, most patients with low hepcidin levels had anemia associated with abnormalities in the gastrointestinal tract, whereas 83.3% patients with high hepcidin levels had iron-refractory iron-deficiency anemia (IRIDA), confirmed by genetic testing. Finally, transferrin/log ferritin ratio accurately identified patients with suppressed hepcidin: AUC 0.98 [95% CI: 0.95–1.02], $p < 0.001$. ***Conclusion:*** OIAT differentiates between classical IDA and other types of anemia caused by abnormalities in iron absorption or systemic iron availability. Additionally, elevated hepcidin in patients with oral iron malabsorption could indicate IRIDA.

© 2023 The Author(s).
Published by S. Karger AG, Basel

Plain Language Summary

Anemia – a decrease in red blood cells that transport oxygen – is a condition that affects a third of the global population and has been associated with increased morbidity. The most common type of anemia is caused by hereditary or acquired changes in iron metabolism, leading to decreased iron availability. Differentiating between the types and causes of iron deficiency is essential for establishing appropriate therapy. Despite the currently used iron status indices, the differentiation between various types of iron-deficiency anemia is still troublesome.

In this retrospective study, we collected data from 39 adult patients who suffered from anemia associated with iron deficiency and had no signs of bleeding or inflammation. We have investigated whether performing an oral iron absorption test and measuring hepcidin, a protein regulating iron metabolism, could aid in establishing a diagnosis that could explain anemia and abnormal iron indices.

karger@karger.com
www.karger.com/aha

© 2023 The Author(s).
Published by S. Karger AG, Basel

This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY) (http://www.karger.com/Services/OpenAccessLicense). Usage, derivative works, and distribution are permitted provided that proper credit is given to the author and the original publisher.

Correspondence to:
Roberta Loveikyte, r.loveikyte@lumc.nl

In this study, we found that 41% of patients had adequate oral iron absorption accompanied by suppressed hepcidin levels, which were suggestive of classical iron-deficiency anemia. In most of these patients, iron-deficiency anemia was attributed to excessive menstrual blood loss. In patients with iron malabsorption, elevated hepcidin levels were suggestive of a genetic disorder known as iron-refractory iron-deficiency anemia. In contrast, patients with lower hepcidin levels were likely to suffer from iron malabsorption not driven by hepcidin but by conditions affecting the stomach, duodenum, or pancreas. This study included a small sample of selected patients; hence, more research is needed to confirm these findings. © 2023 The Author(s).
Published by S. Karger AG, Basel

Introduction

Anemia affects a third of the global population and is associated with increased morbidity and mortality [1–3]. The most common type of anemia is caused by congenital or acquired changes in iron metabolism that lead to decreased iron availability at the site of erythrocyte synthesis [4–6]. The decreased iron availability can result from absolute iron deficiency caused by blood loss, inadequate iron intake or malabsorption, and increased iron needs during pregnancy, which often leads to iron-deficiency anemia (IDA) [1]. On the other hand, decreased iron availability can also result from altered iron distribution – sometimes referred to as functional iron deficiency or iron-restricted anemia – that is commonly associated with inflammation and can lead to anemia of inflammation (AI) [7].

The gold standard for diagnosis of IDA is iron staining in bone marrow aspirate: an invasive test requiring an experienced physician to perform and interpret. Consequently, systemic iron indices – such as ferritin, serum iron, or transferrin – are used in daily practice, but the pitfalls of these indices have been widely documented due to their dual role as acute-phase reactants [8]. Combining classical iron indices can improve diagnostic performance, as it has been shown with the transferrin/log ferritin ratio; however, diagnostic markers reflecting the underlying pathophysiological processes are still warranted to discern different types of anemia [9].

Hepcidin, a systemic iron regulator, is a promising biomarker for diagnosing and treating anemia [10–12]. Hepcidin modulates enteral iron absorption and systemic iron availability by internalizing and degrading ferroportin (FPN), the only known cellular iron exporter [13]. Hepcidin expression is regulated by several stimuli, such as iron deficiency or inflammation [13]. In systemic iron deficiency with intracellular iron depletion, low serum iron and ferritin levels reflect the cellular iron stores. In response to low serum iron, hepcidin expression is suppressed, which leads to increased intestinal iron absorption and iron export from FPN-expressing cells such as enterocytes, hepatocytes, and macrophages. In contrast, proinflammatory cytokines promote hepcidin expression that leads to increased FPN breakdown and iron retention within iron-storing cells, which is reflected by elevated ferritin levels [13]. Abnormalities in hepcidin levels have been observed in different types of anemia and have been associated with oral iron malabsorption [12, 14–17]. Studies have shown that serum hepcidin levels could differentiate IDA from AI in various patient populations [11, 12, 18–25]. Analogous to hepcidin, enteral iron absorption – determined by an oral iron absorption test (OIAT) – was shown to be increased in patients with IDA and diminished in patients with AI compared with healthy volunteers [26].

Unfortunately, approximately one-third of anemia cases are left unexplained, highlighting the need for a better diagnostic process [27]. The diagnostic workup using classical iron parameters is particularly challenging in patients without clinical signs of bleeding or inflammation. In this study, we explored the diagnostic potential of an OIAT in combination with serum hepcidin levels to determine the underlying cause of anemia and abnormal iron status. This study provides insight into how assessing abnormalities in oral iron absorption and hepcidin levels may help guide the physician toward establishing the cause of anemia and appropriate therapy.

Methods

Study Population

Between November 2022 and May 2023, we have conducted an exploratory retrospective study. We have extracted data from medical records of 39 adult patients who were referred to the Department of Internal Medicine at the Haaglanden Medical Center between 2002 and 2019 because of anemia and abnormal iron indices without an identified underlying cause. Nearly all patients had a long-standing anemia and had previously undergone treatment with oral or intravenous iron with varying success; as a result, these patients were referred to a hematologist for further analysis to establish the cause and treatment of anemia. An OIAT and serum hepcidin measurement were not part of a standardized routine care, and these tests were performed at the discretion of a hematologist during outpatient care to guide further diagnostic workup because medical history, physical examination, laboratory tests, and additional investigations (i.e., serological testing for celiac disease, testing for occult fecal blood loss, *Helicobacter pylori* infection or chronic inflammatory diseases) did not yield an explanation. All patients in whom an OIAT and hepcidin tests were performed were included in this study,

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

Table 1. An overview of the study population, stratified by oral iron absorption and hepcidin levels

Patient	Sex	Age	Hemoglobin, mmol/L	MCV, fL	Ferritin, µg/L	Iron, µmol/L	Tsat, %	Tf/log ferritin	Hepcidin, nm	Δ Iron, µmol/L	Diagnosis
Group A: adequate oral iron absorption (Δ iron ≥15 mmol/L) and suppressed hepcidin levels (≤0.5 nM)											
1	F	39	7.8	81	6	11.3	12	4.75	<0.50	44.5	Menorrhagia, *Helicobacter pylori* infection
2	F	48	4.5	67	3	2.2	3	6.92	<0.50	26.4	Menorrhagia
3	M	54	7.6	88	15	6.3	7	3.06	<0.50	75.7	Etiology not established
4	F	52	7.3	74	9	6.0	8	3.14	<0.50	49.6	Etiology not established
5	F	44	4.7	55	3	2.7	3	7.96	<0.50	79.0	GERD with reflux esophagitis
6	F	30	6.2	74	3	4.4	4	8.59	<0.50	23.0	Etiology not established
7	F	40	6.1	73	4	2.5	4	4.65	<0.50	53.1	Menorrhagia
8	F	40	6.7	82	8	5.6	7	3.54	<0.50	24.5	Etiology not established
9	F	66	8.3	87	20	11.8	14	2.54	<0.50	17.7	Etiology not established
10	F	37	6.4	74	3	2.6	4	5.24	<0.50	20.0	Etiology not established
11	F	53	7.0	80	6	7	9	3.86	<0.50	21.9	GERD and uterine myoma
12	F	48	5.7	64	4	3.2	4	4.98	<0.50	47.4	Menorrhagia, α-thalassemia
13	F	49	7.4	95	16	16	24	2.08	<0.50	36.0	Menorrhagia
14	F	37	6.5	82	11	6.3	9	2.69	<0.50	45.6	Menorrhagia
15	F	26	6.4	70	5	–	–	–	<0.50	50.2	Menorrhagia, *Helicobacter pylori* infection
16	F	46	6.4	72	9	4.2	5	3.56	0.5	48.3	Menorrhagia, gastric bypass
Group B: oral iron malabsorption (Δ iron <15 mmol/L) and hepcidin levels below the sex- and age-adjusted median											
17	F	44	7.9	77	9	11.3	17	2.83	<0.50	5.6	Chronic *Helicobacter pylori* gastritis, α-thalassemia
18	F	61	7.1	80	6	5.1	6	4.24	<0.50	5.6	Exocrine pancreatic insufficiency
19	M	83	6.9	82	16	7.2	11	2.16	<0.50	6.0	Exocrine pancreatic insufficiency
20	F	44	5.8	68	3	3.0	3	8.17	<0.50	4.5	*Helicobacter pylori* gastritis
21	F	47	5.7	65	3	3.8	5	6.92	<0.50	7.1	Definite diagnosis not established
22	F	28	7.7	78	6	6.7	9	3.86	<0.50	10.8	Definite diagnosis not established
23	F	45	5.8	–	4	3.9	4	5.98	<0.50	6.5	Autoimmune gastritis
24	F	25	7.4	82	4	11.8	12	6.31	<0.50	11.3	Menorrhagia
25	F	54	7.1	78	6	7.3	9	4.11	0.5	1.8	Exocrine pancreatic insufficiency
26	M	74	8.4	96	18	16	25	2.07	0.8	5.5	Proton-pump inhibitors
27	F	76	7.5	81	15	9.4	14	2.38	0.9	5.7	Definite diagnosis not established
28	F	54	7.2	81	20	10.3	–	–	1.4	2.6	Definite diagnosis not established

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

Table 1 (continued)

Patient	Sex	Age	Hemoglobin, mmol/L	MCV, fL	Ferritin, µg/L	Iron, µmol/L	Tsat, %	Tf/log ferritin	Hepcidin, nm	Δ Iron, µmol/L	Diagnosis
29	F	77	6.7	90	15	9.3	14	2.21	1.9	1.5	Definite diagnosis not established
30	F	69	7.9	79	98	9.9	17	1.21	1.9	0.1	Autoimmune gastritis
31	M	69	7.1	–	30	9.4	18	1.59	1.42	1.6	Autoimmune atrophic gastritis
32	M	55	9.1	90	31	14.0	25	1.48	3.5	9.3	Exocrine pancreatic insufficiency
33	F	43	8.3	88	38	9.5	16	1.52	2.3	6.8	Definite diagnosis not established
Group C: oral iron malabsorption (Δ iron <15 mmol/L) and hepcidin levels above the sex- and age-adjusted median											
34	F	32	7.1	80	57	2.7	4	1.42	5.9	1.3	IRIDA
35	F	37	7.0	76	68	3.6	6	1.36	7.7	4.6	IRIDA
36	M	61	8.7	88	14	5.8	8	2.53	9.3	9.2	Small bowel tumor
37	F	31	6.3	64	47	2.5	4	1.55	9.4	0	IRIDA
38	F	29	6.5	76	29	3.8	6	1.78	13.3	12.2	IRIDA
39	F	38	7.8	77	260	3.6	7	0.87	13.6	9.5	IRIDA

F, female sex; M, male sex; Tsat, transferrin saturation; Tf/log ferritin, transferrin/log10 ferritin ratio; MCV, mean corpuscular volume; GERD, gastroesophageal reflux disease; IRIDA, iron-refractory iron-deficiency anemia. Sex- and age-adjusted median hepcidin levels: 3.6 nM and 4.5 nM for women and men, respectively [28]. Δ Iron is calculated as the difference between the iron value at 120 min and 0 min after ingesting oral iron as part of an OIAT. Exocrine pancreatic insufficiency diagnosis was established by increased fecal elastase excretion, computed tomography, and associated deficiencies in vitamins B12, K, or D. Autoimmune diagnosis was established with serological testing and concurrent vitamin B12 deficiencies. Confirmed mutations in the TMPRSS6 gene established IRIDA diagnosis.

representing a select yet heterogeneous group. The study was approved by the Institutional Review Board Leiden Den Haag Delft (IRB No. N22.072) and has been performed in accordance with the principles of the Declaration of Helsinki (2013).

Data Collection

Demographic, anthropometric, and clinical data (as depicted in Table 1 and online suppl. Table S1; for all online suppl. material, see https://doi.org/10.1159/000535275) were extracted retrospectively from medical records. These data were documented at the time of referral to the Department of Internal Medicine, i.e., 2002–2019. The following biochemical measurements were collected: hemoglobin (Hb), hematocrit, mean corpuscular volume, white blood cell count, platelets, C-reactive protein, erythrocyte sedimentation rate, albumin, estimated glomerular filtration rate, serum iron, ferritin, transferrin, and transferrin saturation (Tsat). If these parameters were not measured on the day of the OIAT, the biochemical measurements were extracted from a date closest to the OIAT. In all but one case, the parameters were available within 3 months from the OIAT. Hepcidin levels were also extracted from the medical records that, in most cases, were measured on the same day as the OIAT. In addition, the transferrin/$\log_{10}$ ferritin ratio (Tf/log ferritin) was calculated [9]. Data regarding all performed diagnostic tests during routine care and the final diagnosis were also collected, as described in the following sections.

Study Definitions

Anemia was defined as Hb <7.5 mmol/L (<120.7 g/L) for females or <8.5 mmol/L (<137.0 g/L) for males, based on the Dutch national reference values [29]. There is no widely used and standardized protocol for an OIAT [30, 31]. The data extracted for this study were from an OIAT that was performed using 400 mg of immediate-release ferrous fumarate – equivalent to 130 mg of elemental iron – taken as a single dose on an empty stomach after an overnight fast. Blood was drawn just before ingestion of iron tables (i.e., at 0 min) and 30, 60, 90, and 120 min after ingesting ferrous fumarate. Over the course of the OIAT, patients were not allowed to consume food. The difference between serum iron value (Δ iron) at 120 and 0 min was calculated; oral iron malabsorption was defined as Δ iron <15 µmol/L [26, 32].

In this study, hepcidin-driven iron malabsorption was considered in cases with hepcidin levels above the sex- and age-adjusted median, which was established in the Dutch population: 3.6 nM and 4.5 nM for women and men, respectively [28]. Hepcidin levels below the sex- and age-adjusted median were considered as potentially inappropriate suppression in cases of oral iron malabsorption. A hepcidin level ≤0.5 nM was considered suppressed and characteristic of classical IDA, since it is characterized by hepcidin levels below the detection limit [11, 33, 34].

Diagnostic Process

All relevant information regarding the diagnostic process and final clinical diagnoses after the OIAT and hepcidin measurement were also extracted from medical records. The diagnostic workup performed by a hematologist has been depicted in Figure 1. While the diagnostic workup was not a part of an established study protocol, it was, nonetheless, consistent. Patients with adequate oral iron absorption, especially in association with suppressed hepcidin levels, were diagnosed as having IDA. Patients with oral

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

iron malabsorption and a hepcidin level below the sex- and age-adjusted median were examined for *H. pylori* and autoimmune gastritis, if these tests were not performed before. In patients with additional deficiencies, celiac disease was excluded a second time; exocrine pancreas function was evaluated by the fecal elastase test with or without computer tomography. Endoscopic examination, fecal calprotectin, and fecal examination for helminth infections were performed on indication. Finally, patients with oral iron malabsorption and a hepcidin level above the sex- and age-adjusted median underwent analysis for TMPRSS6 gene mutations, which could indicate iron-refractory iron-deficiency anemia (IRIDA) since it is a likely diagnosis for hepcidin-driven iron malabsorption in the absence of inflammation [32].

Statistical Analysis

Descriptive data are reported as median with interquartile ranges (IQR: Q1–Q3) for continuous variables. Categorical variables are presented as proportions: absolute numbers of available data (n) with corresponding percentages (%). Normality assessment was performed by visually examining normal probability plots (Q-Q) and histograms. Mann-Whitney U and χ^2 tests were used as appropriate to compare independent subgroups. Spearman's rank correlation coefficients (ρ) were used to determine correlations between variables. In addition, receiver-operating characteristic (ROC) analysis with the area under the curve (AUC) as an overall measure of fit was used to evaluate the discriminative capacity of different biochemical parameters to detect oral iron malabsorption and suppressed hepcidin levels. Two-tailed p values <0.05 were considered statistically significant; the Benjamini-Hochberg procedure was used to adjust for multiple testing, considering significance under a false discovery rate of 5%. Statistical analysis was performed with the SPSS Statistics 25 software package (SPSS Inc., Chicago, IL, USA). The proportion of available data is presented in online supplementary Table S1.

Results

Study Population

Thirty-nine (39) adult patients were included in the study, as shown in online supplementary Table S1. Patients were primarily female (84.6%) and, on average, 46 years old. The median Hb was 7.10 mmol/L (IQR: 6.40–7.70 mmol/L), serum iron 6.15 μmol/L (IQR: 3.60–9.60 μmol/L), ferritin 9.00 μg/L (IQR: 4.00–20.00 μg/L), and Tsat 8.00% (IQR: 4.00–14.00%). Hepcidin was suppressed in 64.1% of patients. Six patients (15.4%) had hepcidin levels above the sex- and age-adjusted median (online suppl. Table S1).

Patient Characterization Based on OIAT and Hepcidin Tests

Table 1 presents an overview of patients stratified by oral iron absorption and hepcidin levels. Group A includes 16 patients with adequate iron absorption (Δ iron ≥15 μmol/L) and suppressed hepcidin levels; only 1 patient in this group was male. The median Hb was 6.45 mmol/L (IQR: 6.13–7.38 mmol/L), serum iron 5.60 μmol/L (IQR: 2.70–7.00 μmol/L), ferritin 6.00 μg/L (IQR: 3.25–10.50 μg/L), and Tsat 7.00% (IQR: 4.00–9.00%). All patients in Group A were diagnosed with classical IDA; menorrhagia was the main cause in 50% of cases, especially after *H. pylori* eradication. Despite extensive diagnostic investigations, the cause for IDA was not found in 6 patients.

More than half of the patients (n = 23) were considered to have oral iron malabsorption (Δ iron <15 μmol/L); 18 of these patients were female. These patients were further stratified by hepcidin levels into Group B and Group C. Group B includes patients with hepcidin levels below the sex- and age-adjusted median (n = 17). In this group, iron deficiency was associated with conditions affecting the stomach, duodenum, or pancreas. In 6 patients, the IDA was attributed to autoimmune gastritis, *H. pylori* infection, or proton-pump inhibitor use. In 4 patients, exocrine pancreas insufficiency was considered the cause of iron deficiency and other accompanying nutritional deficiencies, i.e., vitamin B12, K, and/or D. Only 1 patient within Group B had anemia attributed to menorrhagia. A definite cause for abnormal iron status could not be identified in 6 patients, as shown in Table 1.

Patients with oral iron malabsorption and hepcidin levels above the sex- and age-adjusted median were allocated to Group C (Table 1). In contrast to Group B, iron malabsorption in Group C was considered to be driven by hepcidin. All but 1 patient within this group had one or more mutations in the TMPRSS6 gene encoding matriptase protein, leading to an inappropriate increase in hepcidin levels, a condition known as IRIDA.

Comparing patients in Group A and Group B revealed no significant differences after correction for multiple testing, as shown in Table 2. In short, classical iron indices alone are unlikely to accurately differentiate between patients with adequate and inadequate iron absorption.

Differences between Patients with Oral Iron Malabsorption, Stratified by Hepcidin Levels

Table 3 presents differences between patients in Group B and Group C. Patients with oral iron malabsorption attributed to conditions affecting the stomach, duodenum, or pancreas (Group B) had significantly higher serum iron than patients with hepcidin-driven malabsorption (Group C): median 9.40 μmol/L (IQR: 5.90–10.80 μmol/L) versus

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

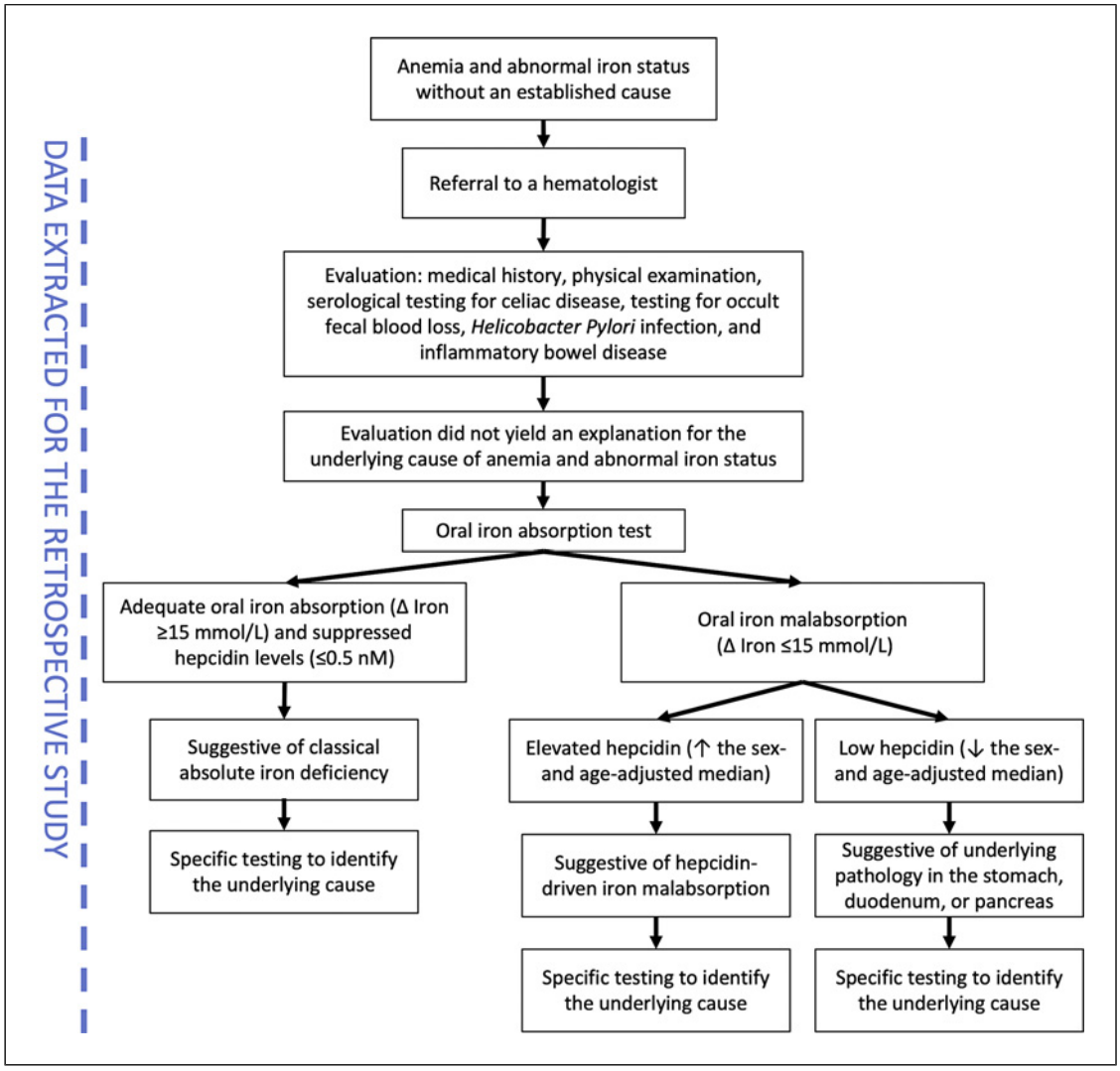

Fig. 1. Overview of the diagnostic process performed by the physicians during routine care and the data extracted for the retrospective study. Median sex- and age-adjusted hepcidin level in the Dutch population 3.6 nM for women and 4.5 nM for men. Δ Iron is calculated as the difference between the serum iron value at 120 min and 0 min after ingesting oral iron as part of an OIAT.

3.60 μmol/L (IQR: 2.65–4.30 μmol/L) in Group B and Group C, respectively; $p < 0.01$. Other iron parameters, such as Tf/log ferritin ratio, were also higher in Group B, but these differences were not significant after correction for multiple testing.

Classical Iron Parameters as Potential Biomarkers for Abnormalities in Hepcidin and OIAT

Strong associations between hepcidin and iron indices have been observed: $\rho = 0.767$ ($p < 0.001$) and $\rho = -0.768$ ($p < 0.001$) for ferritin and Tf/log ferritin, respectively. All correlations between hepcidin, iron absorption, and other parameters are depicted in online supplementary Table S2. ROC analysis with corresponding AUC for suppressed hepcidin levels was performed using iron indices strongly associated with hepcidin. Both ferritin and Tf/log ferritin accurately identified suppressed hepcidin levels; however, Tf/log ferritin was superior to ferritin alone: AUC 0.98 (95% CI: 0.956–1.02), $p < 0.001$ (Table 4). For Tf/log ferritin, a cutoff point of 2.11 had a 96% sensitivity and a 77% specificity, where patients with values higher than the cutoff point were likely to have suppressed hepcidin levels.

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

Table 2. Differences between patients with hepcidin levels below the age- and sex-adjusted median, stratified by oral iron absorption

Variables	OIAT ≥15 (n = 16)	OIAT <15 (n = 17)	p value[a]
Age at referral	45.00 (37.50–51.25)	54.00 (44.00–71.50)	<0.05
Hemoglobin, mmol/L	6.45 (6.13–7.38)	7.20 (6.80–7.90)	NS
Hematocrit, %	0.34 (0.32–0.38)	0.38 (0.33–0.41)	NS
MCV, fL	74.00 (70.50–82.00)	81.00 (78.00–88.00)	NS
WBC, $\times 10^9$	5.55 (4.75–6.38)	7.30 (5.80–8.70)	<0.05
Platelets, $\times 10^9$	218.00 (177.25–245.00)	269.00 (187.25–283.50)	NS
ESR, mm/h	17.00 (8.50–23.50)	11.50 (6.50–16.75)	NS
Ferritin, µg/L	6.00 (3.25–10.50)	15.00 (5.00–25.00)	NS
Iron, µmol/L	5.60 (2.70–7.00)	9.40 (5.90–10.80)	<0.05
Tsat, %	7.00 (4.00–9.00)	13.00 (6.75–17.00)	<0.05
Transferrin/log ferritin	3.86 (3.06–5.24)	2.61 (1.66–5.54)	NS

Data are presented as means ± standard deviation (SD), median [IQR], or proportions of available data (n) with corresponding percentages (%). Age- and sex-adjusted median hepcidin levels: 3.6 nM and 4.5 nM for women and men, respectively [28]. OIAT, oral iron absorption test (Δ iron is calculated as the difference between the iron value at 120 min and 0 min after ingesting oral iron as part of an OIAT). MCV, mean corpuscular volume; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; Tsat, transferrin saturation. NS, statistically nonsignificant. Two-tailed p values <0.05 were considered statistically significant; the Benjamini-Hochberg procedure was used to adjust for multiple testing, adopting a 5% false discovery rate (FDR). [a]None of the p values were statistically significant after adjustment for multiple testing.

Table 3. Differences between patients with iron malabsorption, stratified by hepcidin levels below or above the age- and sex-adjusted median

Variables	Hepcidin < median (n = 17)	Hepcidin > median (n = 6)	p value
Age at referral	54.00 (44.00–71.50)	34.50 (30.50–43.75)	<0.05
Hemoglobin, mmol/L	7.20 (6.80–7.90)	7.05 (6.45–8.03)	NS
Hematocrit, %	0.38 (0.33–0.41)	0.36 (0.33–0.41)	NS
MCV, fL	81.00 (78.00–88.00)	76.50 (73.00–82.00)	NS
WBC, $\times 10^9$	7.30 (5.80–8.70)	7.95 (4.08–9.83)	NS
Platelets, $\times 10^9$	269.00 (187.25–283.50)	369.50 (280.00–473.00)	<0.05
ESR, mm/h	11.50 (6.50–16.75)	12.00 (9.50–23.00)	NS
Ferritin, µg/L	15.00 (5.00–25.00)	52.00 (25.25–116.00)	<0.05
Iron, µmol/L	9.40 (5.90–10.80)	3.60 (2.65–4.30)	**<0.01**[a]
Tsat, %	13.00 (6.75–17.00)	6.00 (4.00–7.25)	<0.05
Transferrin/log ferritin	2.61 (1.66–5.54)	1.49 (1.24–1.97)	<0.05
Δ Iron, µmol/L	5.60 (2.20–6.95)	6.90 (0.95–10.18)	NS

Data are presented as means ± standard deviation (SD), median [IQR], or proportions of available data (n) with corresponding percentages (%). Age- and sex-adjusted median hepcidin levels: 3.6 nM and 4.5 nM for women and men, respectively [28]. MCV, mean corpuscular volume; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; Tsat, transferrin saturation. Δ Iron is calculated as the difference between the iron value at 120 min and 0 min after ingesting oral iron as part of an OIAT. NS, statistically nonsignificant. Two-tailed p values <0.05 were considered statistically significant; the Benjamini-Hochberg procedure was used to adjust for multiple testing, adopting a 5% false discovery rate (FDR). [a]p value represents statistical significance after adjustment for multiple testing.

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

Table 4. Biomarker discriminative capacity for suppressed hepcidin levels and oral iron malabsorption

	Suppressed hepcidin		
	AUC	95% CI	*p* value
Ferritin, µg/L	0.96	(0.92–1.01)	<0.001
Transferrin/log ferritin	0.98	(0.95–1.02)	<0.001
	Iron malabsorption		
	AUC	95% CI	*p* value
Ferritin, µg/L	0.76	(0.61–0.91)	<0.001
Hepcidin, nm	0.81	(0.68–0.95)	<0.001
Transferrin/log ferritin	0.76	(0.60–0.91)	<0.01

AUC, area under the curve; 95% CI, 95% confidence interval.

In addition, ROC analysis with corresponding AUC for oral iron malabsorption was performed. All analyzed variables – ferritin, hepcidin, and Tf/log ferritin – showed a better discriminative capacity for iron malabsorption than random chance; however, hepcidin was the most accurate (AUC: 0.81, 95% CI: 0.68–0.95, $p < 0.001$), as depicted in Table 4.

Discussion

In this retrospective study, we assessed the diagnostic utility of an OIAT and hepcidin in a select group of patients with anemia and abnormal iron status without overt signs indicative of an underlying disorder. We found that 41% of patients had adequate oral iron absorption accompanied by suppressed hepcidin levels, which was compatible with classical IDA. All other patients had oral iron malabsorption, indicating its prominent role in the development of iron deficiency. Most patients with oral iron malabsorption and hepcidin levels below the sex- and age-adjusted median had anemia caused by conditions affecting the stomach, duodenum, or pancreas. In contrast, 5 of the 6 (83.3%) of patients with oral iron malabsorption and hepcidin levels above the age- and sex-adjusted median had IRIDA. In short, hepcidin could be a useful diagnostic test in patients with abnormal OIAT to determine whether anemia is hepcidin-driven or likely related to abnormalities in the stomach, duodenum, or pancreas. Last but not least, we found that in environments with fewer resources, Tf/log ferritin higher than 2.11 could be used to identify patients with suppressed hepcidin. In conclusion, primarily OIAT rather than hepcidin levels could aid physicians in discerning classical IDA from other types of anemia in patients without overt signs of bleeding or inflammation.

Patients with adequate OIAT were most likely to have IDA, as classical IDA is caused by decreased systemic iron availability rather than abnormalities in iron metabolism. In line with previous studies, all patients with adequate OIAT had suppressed hepcidin levels, representing a physiological response to restore serum iron by increasing intestinal iron absorption [33, 34]. Therefore, patients in these cases can be treated with oral iron therapy due to amplified intestinal iron absorption. In contrast to patients with adequate OIAT, patients with oral iron malabsorption had either suppressed, detectable, or even elevated levels of hepcidin. In short, we found that primarily OIAT rather than hepcidin or classical iron indices alone can discern classical IDA from other types of anemia in patients without overt bleeding or inflammation.

Hepcidin levels can guide further diagnostic processes in patients with established oral iron malabsorption. Five out of 6 patients with hepcidin levels above the sex- and age-adjusted median and oral iron malabsorption had IRIDA, a genetic disorder resulting from mutations in the TMPRSS6 gene, which leads to uncontrolled hepcidin expression that drives oral iron malabsorption [32]. In cases of hepcidin-driven malabsorption, oral iron therapy is unlikely to be effective and these patients should be treated with intravenous iron; some patients might need repeated infusions. Conversely, in patients with oral iron malabsorption and hepcidin levels below the sex- and age-adjusted, anemia was caused by a decrease in systemic iron availability either due to inflammation, such as *H. pylori* infection, or abnormalities in iron uptake resulting from higher local pH that impairs the dissociation of iron from its carrier and the uptake into enterocytes, as is the case in atrophic gastritis or proton-pump inhibitor use [35]. In these cases, abnormalities in hepcidin are not the driving cause but rather a consequence of anemia; therefore, it is unlikely that only iron replacement will adequately manage anemia in these patients. The primary therapeutic goal should be to address the underlying cause of malabsorption – such as inflammation – before prescribing iron therapy. To summarize, patients with abnormal OIAT and elevated hepcidin are likely to suffer from IRIDA, whereas patients with abnormal OIAT and lower hepcidin levels are likely to have

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

conditions affecting the stomach, duodenum, or pancreas.

Moreover, we observed an unexpected relationship between exocrine pancreatic insufficiency and iron status. Four out of 17 patients with oral iron malabsorption had anemia associated with exocrine pancreatic insufficiency and concurrent nutritional deficiencies in vitamin B12, D, and/or K. There is a lack of literature regarding this association; most available studies were performed before the 1990s and showed iron overload in cases of pancreatic disease, but the results were inconsistent, given that a high dose of pancreatic enzymes facilitated iron uptake [36, 37]. Sabor et al. [38] hypothesized that iron deficiency might be related to premucosal or mucosal pathology in cases of exocrine pancreatic insufficiency. Inadequate food digestion and the uptake of fat-soluble vitamins (e.g., vitamin D) can play a crucial role in modulating hepcidin levels and the proliferation of erythroid progenitor cells, which might explain why patients with exocrine pancreatic insufficiency often suffer from anemia and iron deficiency [39–41]. In addition, membrane proteins – hephaestin and divalent metal transporter 1 – involved in oral iron absorption and systemic availability have also been found in pancreatic cells, suggesting that the pancreas might play a significant role in iron metabolism [42, 43]. We postulate that oral iron malabsorption in cases of exocrine pancreatic insufficiency could be related to a decrease in pancreatic enzymes that might impair iron uptake at the luminal side of enterocytes. However, the relationship between iron absorption and pancreatic function necessitates further investigation.

Lastly, OIAT is a simple test that could be done in most settings with access to oral iron and venipuncture for serum iron measurements. If necessary, OIAT could be simplified to venipuncture just before and 120 min after ingestion of oral iron. While we observed good discriminative capacity of hepcidin, ferritin, and Tf/log ferritin to identify oral iron malabsorption, we do not suggest using these parameters instead of an OIAT, given that oral iron malabsorption might not be accurately identified in patients with low hepcidin levels. In contrast to an OIAT, hepcidin measurement might not be available in environments with fewer resources. In these cases, Tf/log ferritin could be used instead since it was strongly negatively associated with hepcidin and showed an excellent discriminative capacity to indicate patients with suppressed hepcidin levels. However, prospective studies with larger sample sizes and a more generalizable study population are necessary to validate our findings and to establish a validated cutoff point for Tf/log ferritin or even ferritin.

This study is the first to evaluate the diagnostic utility of OIAT and hepcidin for discerning classical IDA from other underlying pathologies in patients without overt signs of bleeding or inflammation. However, this exploratory retrospective study has several limitations. The main limitation is that the decision to perform an OIAT, measure hepcidin, and the type of subsequent diagnostic workup was made by treating physicians based on clinical grounds, which might have caused potential selection bias. In addition, the diagnostic workup performed by the physicians was not standardized and there was a lack of essential data, such as dietary habits or a validated questionnaire regarding menstrual blood loss. Therefore, this select group is largely overrepresented by young females and the results might not apply to patients with chronic inflammatory conditions. This study has also been limited by a lack of clearly defined iron restriction, absolute iron deficiency, as well as the absence of blood loss and inflammation, given that they were established primarily based on clinical evaluation. Finally, a larger sample size would have allowed us to establish more reliable subgroup analyses.

Despite the limitations, this study shows that primarily an OIAT rather than hepcidin could aid physicians in differentiating classical IDA from other types of anemia caused by abnormalities in enteral iron absorption or systemic iron availability. In patients with anemia and abnormal iron status without an apparent cause, an OIAT in combination with hepcidin or Tf/log ferritin may be helpful diagnostic tools to identify the underlying cause of anemia, tailor further diagnostic workup, and establish appropriate therapy. Nevertheless, prospective studies are necessary to validate this diagnostic approach and to establish hepcidin and Tf/log ferritin cutoff points regarding oral iron malabsorption. Finally, the relationship between pancreatic function, hepcidin, and oral iron malabsorption needs to be explored further.

Statement of Ethics

The study was approved by the Institutional Review Board Leiden Den Haag Delft (IRB No. N22.072) and the Research Bureau at the Haaglanden Medical Center (No. 2022-025); an exemption for informed consent was granted. The study was conducted in accordance with the principles of the Declaration of Helsinki (2013).

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

Conflict of Interest Statement

A.E.v.d.M.-d.J. received unrestricted research grants from Galapagos, Norgine, Vedanta, and Nestle, including speaker's fees from Galapagos, Tramedico, Takeda, Ferring, and Janssen Pharmaceuticals. R.L. has served on the advisory board of Cablon Medical and received travel expenses from Galapagos as well as from Cablon Medical. All other authors have no conflicts of interest.

Funding Sources

The research position of R.L. was supported by the Dutch research consortium Initiative on Crohn and Colitis (ICC) and Cablon Medical. The sponsoring bodies had no role in the conceptualization or design of the study, including data collection, analysis, data interpretation, or manuscript writing.

Author Contributions

R.L., A.E.v.d.M.-d.J., and L.T.V. conceptualized and designed the study. Y.v.d.B., A.E.v.d.M.-d.J., and L.T.V. were responsible for resource acquisition. R.L. and Y.v.d.B. collected study data. R.L. performed data analysis and data visualization under the supervision of A.E.v.d.M.-d.J. and L.T.V. R.L. wrote the first draft of the manuscript. All authors contributed to the manuscript revision, and read and approved the final version to be submitted for publication.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

References

1 Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. Lancet. 2021;397(10270):233–48.
2 WHO. Global nutrition targets 2025: anaemia policy brief (WHO/NMH/NHD/14.4). 2014; Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-14.4.
3 Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;123(5):615–24.
4 Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet. 2016;387(10021):907–16.
5 Sun H, Weaver CM. Decreased iron intake parallels rising iron deficiency anemia and related mortality rates in the US population. J Nutr. 2021;151(7):1947–55.
6 Schop A, Stouten K, Riedl JA, van Houten RJ, Leening MJG, van Rosmalen J, et al. A new diagnostic work-up for defining anemia etiologies: a cohort study in patients ≥50 years in general practices. BMC Fam Pract. 2020;21(1):167.
7 Gangat N, Wolanskyj AP. Anemia of chronic disease. Semin Hematol. 2013;50(3):232–8.
8 Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. Biochim Biophys Acta. 2010;1800(8):760–9.
9 Castel R, Tax MGHM, Droogendijk J, Leers MPG, Beukers R, Levin MD, et al. The transferrin/log(ferritin) ratio: a new tool for the diagnosis of iron deficiency anemia. Clin Chem Lab Med. 2012;50(8):1343–9.
10 Ganz T. Anemia of inflammation. N Engl J Med. 2019;381(12):1148–57.
11 Girelli D, Nemeth E, Swinkels DW. Hepcidin in the diagnosis of iron disorders. Blood. 2016;127(23):2809–13.
12 Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105(2):260–72.
13 Roth MP, Meynard D, Coppin H. Regulators of hepcidin expression. Vitam Horm. 2019;110:101–29.
14 Donker AE, Raymakers RAP, Vlasveld LT, van Barneveld T, Terink R, Dors N, et al. Practice guidelines for the diagnosis and management of microcytic anemias due to genetic disorders of iron metabolism or heme synthesis. Blood. 2014;123(25):3873–86; quiz 4005.
15 Pagani A, Nai A, Silvestri L, Camaschella C. Hepcidin and anemia: a tight relationship. Front Physiol. 2019;10:1294.
16 Bregman DB, Morris D, Koch TA, He A, Goodnough LT. Hepcidin levels predict nonresponsiveness to oral iron therapy in patients with iron deficiency anemia. Am J Hematol. 2013;88(2):97–101.
17 Stoffel NU, von Siebenthal HK, Moretti D, Zimmermann MB. Oral iron supplementation in iron-deficient women: how much and how often? Mol Aspects Med. 2020;75:100865.
18 van Santen S, van Dongen-Lases EC, de Vegt F, Laarakkers CMM, van Riel CM, van Ede AE, et al. Hepcidin and hemoglobin content parameters in the diagnosis of iron deficiency in rheumatoid arthritis patients with anemia. Arthritis Rheum. 2011;63(12):3672–80.
19 Bergamaschi G, Di Sabatino A, Albertini R, Costanzo F, Guerci M, Masotti M, et al. Serum hepcidin in inflammatory bowel diseases: biological and clinical significance. Inflamm Bowel Dis. 2013;19(10):2166–72.
20 Shu T, Jing C, Lv Z, Xie Y, Xu J, Wu J. Hepcidin in tumor-related iron deficiency anemia and tumor-related anemia of chronic disease: pathogenic mechanisms and diagnosis. Eur J Haematol. 2015;94(1):67–73.
21 Pasricha SR, Atkinson SH, Armitage AE, Khandwala S, Veenemans J, Cox SE, et al. Expression of the iron hormone hepcidin distinguishes different types of anemia in African children. Sci Transl Med. 2014;6(235):235re3.
22 Lasocki S, Baron G, Driss F, Westerman M, Puy H, Boutron I, et al. Diagnostic accuracy of serum hepcidin for iron deficiency in critically ill patients with anemia. Intensive Care Med. 2010;36(6):1044–8.
23 Galetti V, Stoffel NU, Sieber C, Zeder C, Moretti D, Zimmermann MB. Threshold ferritin and hepcidin concentrations indicating early iron deficiency in young women based on upregulation of iron absorption. EClinicalMedicine. 2021;39:101052.
24 Baart AM, van Noord PAH, Vergouwe Y, Moons KGM, Swinkels DW, Wiegerinck ET, et al. High prevalence of subclinical iron deficiency in whole blood donors not deferred for low hemoglobin. Transfusion. 2013;53(8):1670–7.
25 Aksan A, Wohlrath M, Iqbal TH, Farrag K, Dignass A, Stein J. Serum hepcidin levels predict intestinal iron absorption in patients with inflammatory bowel disease. Clin Lab. 2019;65(3).
26 Kobune M, Miyanishi K, Takada K, Kawano Y, Nagashima H, Kikuchi S, et al. Establishment of a simple test for iron absorption from the gastrointestinal tract. Int J Hematol. 2011;93(6):715–9.
27 Guralnik J, Ershler W, Artz A, Lazo-Langner A, Walston J, Pahor M, et al. Unexplained anemia of aging: etiology, health consequences, and diagnostic criteria. J Am Geriatr Soc. 2022;70(3):891–9.
28 Galesloot TE, Vermeulen SH, Geurts-Moespot AJ, Klaver SM, Kroot JJ, van Tienoven D, et al. Serum hepcidin: reference ranges and biochemical correlates in the general population. Blood. 2011;117(25):e218–25.

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024

29 Hooijkaas HMK, Souverijn JHM, Smeets LC. Tax GHM Handboek medische laboratoriumdiagnostiek. 2 ed. Houten: Prelum Uitgevers; 2013.
30 Jensen NM, Brandsborg M, Boesen AM, Yde H, Dahlerup JF. Low-dose oral iron absorption test: establishment of a reference interval. Scand J Clin Lab Invest. 1998;58(6):511–9.
31 Joosten E, Vander Elst B, Billen J. Small-dose oral iron absorption test in anaemic and non-anaemic elderly hospitalized patients. Eur J Haematol. 1997;58(2):99–103.
32 Donker AE, Schaap CCM, Novotny VMJ, Smeets R, Peters TMA, van den Heuvel BLP, et al. Iron refractory iron deficiency anemia: a heterogeneous disease that is not always iron refractory. Am J Hematol. 2016;91(12):82–90.
33 Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112(10):4292–7.
34 Kroot JJ, Laarakkers CMM, Geurts-Moespot AJ, Grebenchtchikov N, Pickkers P, van Ede AE, et al. Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism disorders. Clin Chem. 2010;56(10):1570–9.
35 Cavalcoli F, Zilli A, Conte D, Massironi S. Micronutrient deficiencies in patients with chronic atrophic autoimmune gastritis: a review. World J Gastroenterol. 2017;23(4):563–72.
36 Kavin H, Charlton RW, Jacobs P, Green R, Torrance JD, Bothwell TH. Effect of the exocrine pancreatic secretions on iron absorption. Gut. 1967;8(6):556–64.
37 Sinniah R, Bell TK, Neill DW. The effect of pancreatectomy and other agents on iron absorption and storage in the rat. J Clin Pathol. 1973;26(2):130–7.
38 Saboor M, Zehra A, Qamar K, Moinuddin. Disorders associated with malabsorption of iron: a critical review. Pak J Med Sci. 2015;31(6):1549–53.
39 Zughaier SM, Alvarez JA, Sloan JH, Konrad RJ, Tangpricha V. The role of vitamin D in regulating the iron-hepcidin-ferroportin axis in monocytes. J Clin Transl Endocrinol. 2014;1(1):19–25.
40 Alon DB, Chaimovitz C, Dvilansky A, Lugassy G, Douvdevani A, Shany S, et al. Novel role of 1,25(OH)(2)D(3) in induction of erythroid progenitor cell proliferation. Exp Hematol. 2002;30(5):403–9.
41 Smith EM, Alvarez JA, Kearns MD, Hao L, Sloan JH, Konrad RJ, et al. High-dose vitamin D(3) reduces circulating hepcidin concentrations: a pilot, randomized, double-blind, placebo-controlled trial in healthy adults. Clin Nutr. 2017;36(4):980–5.
42 Koch RO, Zoller H, Theuri I, Obrist P, Egg G, Strohmayer W, et al. Distribution of DMT 1 within the human glandular system. Histol Histopathol. 2003;18(4):1095–101.
43 Hudson DM, Curtis SB, Smith VC, Griffiths TAM, Wong AYK, Scudamore CH, et al. Human hephaestin expression is not limited to enterocytes of the gastrointestinal tract but is also found in the antrum, the enteric nervous system, and pancreatic {beta}-cells. Am J Physiol Gastrointest Liver Physiol. 2010;298(3):G425–32.

Downloaded from http://karger.com/aha/article-pdf/doi/10.1159/000535275/4053834/000535275.pdf by guest on 08 April 2024