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Hepcidin and Iron Status in Patients With Inflammatory Bowel Disease Undergoing Induction Therapy With Vedolizumab or Infliximab

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Background: Hepcidin, the systemic iron regulator, could be critical in differentiating iron deficiency (ID) from functional iron restriction in inflammatory bowel disease (IBD). We assessed hepcidin as a diagnostic ID marker and explored the relationship between hepcidin and its regulators in patients with IBD undergoing induction therapy with infliximab (IFX) or vedolizumab (VEDO).

Methods: Patients with active IBD receiving induction therapy with IFX or VEDO were included. Serum samples at baseline and after 6 weeks of induction therapy were analyzed for hepcidin, inflammation- and hypoxia-associated cytokines, and oxidative stress. Data were analyzed by stratifying based on the response at week 14. Results were compared with samples from age- and sex-matched healthy control subjects.

Results: Patients receiving induction therapy with IFX (n = 71) or VEDO (n = 51) and healthy control subjects (n = 50) were included. At baseline, hepcidin correlated positively with ferritin and negatively with soluble transferrin receptor/log ferritin index ($P < .001$). ID was prevalent in 96.7% of patients who had hepcidin levels below the median. Hepcidin accurately identified ID: the area under the curve (hepcidin) was 0.89 (95% confidence interval, 0.82-0.95; $P < .001$). In total, 75.4% of patients responded to induction therapy; inflammation, hepcidin, and ferritin decreased significantly, while transferrin increased during induction therapy. These changes were observed only in patients who responded to the therapy.

Conclusions: Hepcidin levels in IBD are primarily determined by ID, even in an inflammatory state. In addition, induction therapy can decrease hepcidin levels, which might lead to better bioavailability of iron supplements. Therefore, hepcidin is a potential diagnostic ID biomarker that could assist therapeutic decision making.

Lay Summary

Absolute iron deficiency is the primary determinant of hepcidin levels, even in an inflammatory state. Induction therapy can decrease hepcidin levels, which might improve iron bioavailability. Hence, hepcidin is a potential diagnostic iron deficiency biomarker that could assist therapeutic decision making.

Key Words: hepcidin, inflammatory bowel disease, iron deficiency, iron deficiency anemia, biomarkers.

Introduction

Iron deficiency (ID) is a prominent issue in patients with inflammatory bowel disease (IBD). ID is prevalent in up to 90% of patients with IBD and is characterized by low iron stores that eventually lead to iron-deficient erythropoiesis and anemia, which is associated with worse disease outcomes, reduced quality of life, and increased healthcare costs.[1-3] Chronic intestinal blood loss, poor iron intake or malabsorption, inflammation-mediated systemic iron restriction, and myelosuppression predispose patients with IBD to anemia, absolute ID, and functional ID. These predisposing factors render the diagnosis and treatment of ID and anemia challenging in patients with IBD.[4] The multifactorial etiology of anemia in patients with IBD often involves a combination of true ID anemia (IDA) and anemia of chronic disease (ACD). The gold standard for ID diagnosis is iron staining in bone marrow aspirates: an invasive and labor-intensive procedure rarely performed in routine clinical practice. Hence, ID is routinely assessed using systemic biomarkers such as ferritin or transferrin. These iron indices are affected by inflammation

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Key Messages

- **What is already known?**

Hepcidin is a systemic iron regulator. Elevated hepcidin levels are associated with iron malabsorption and systemic iron restriction, which can prolong iron therapy or lead to iron-deficient erythropoiesis.

- **What is new here?**

Absolute iron deficiency is the primary determinant of hepcidin levels, even in an inflammatory state. In addition, anti-inflammatory therapy can reduce hepcidin levels, which might lead to better iron bioavailability.

- **How can this study help patient care?**

Hepcidin levels can indicate absolute iron deficiency and differentiate it from iron restriction, which aids in prescribing the appropriate therapy.

complicating the interpretation of iron stores.[5] Nevertheless, accurate differentiation of absolute ID from functional ID is imperative for choosing the appropriate treatment.

Hepcidin—the systemic iron regulator—could be a potential biomarker for distinguishing absolute from functional ID. Hepcidin modulates enteral iron absorption and systemic iron availability by altering the expression of cellular iron exporter ferroportin. Hepcidin internalizes and degrades ferroportin, making it impossible for absorbed enteral iron, recycled iron in macrophages, or stored iron in hepatocytes to be released into the circulation for further use (Figure 1).[6] Multiple factors regulate the expression of hepcidin: inflammation and iron overload increase hepcidin, whereas hypoxia, increased erythropoiesis, and ID decrease hepcidin (Figure 1). Multiple studies, primarily small observational studies, addressed hepcidin levels in patients with IBD with inconsistent findings: some showed higher and some lower hepcidin levels in patients with IBD than in healthy control subjects.[7-12] In contrast, higher hepcidin levels were consistently associated with iron malabsorption in patients with IBD.[8,13,14] To our knowledge, researchers have never investigated hepcidin levels in patients with IBD in regard to different regulatory stimuli such as hypoxia or erythropoiesis.

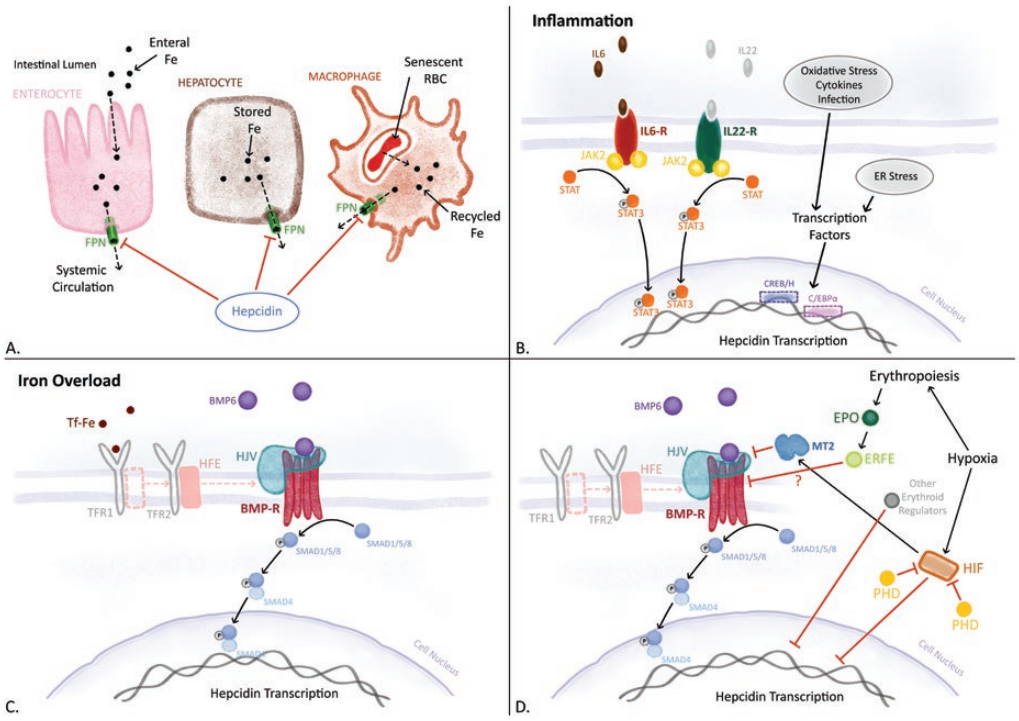

Figure 1. Regulation of hepcidin and iron availability. A, Hepcidin regulates systemic iron availability and enteral iron absorption by internalizing and degrading intracellular iron exporter ferroportin (FPN). An increase in hepcidin leads to reduced FPN levels that prevent enteral iron (Fe) from duodenal enterocytes, stored iron from hepatocytes, and recycled iron from senescent red blood cells (RBCs) in macrophages from export to the systemic circulation, which leads to iron restriction and functional iron deficiency. B, In cases of inflammation, interleukin (IL)-6 and IL-22 bind to their receptors that activate JAK2 and lead to phosphorylation of STAT3. Phosphorylated STAT3 then translocates to the cell nucleus and upregulates hepcidin by inducing transcription of the *HAMP* gene. In addition, other cytokines such as IL-1β, oxidative stress, endoplasmic reticulum (ER) stress, and infectious inflammation upregulate hepcidin by inducing transcription factors such as the CREBH or C/EBPα. C, In cases of iron overload, bone morphogenetic protein 6 (BMP6) binds to the BMP receptor (BMP-R) and coreceptor hemojuvelin (HJV). This leads to the phosphorylation of SMAD1/5/8, which binds with SMAD4 and translocates to the cell nucleus inducing *HAMP* transcription. It has been shown that the transferrin receptor 2 (TFR2) complex with human hemochromatosis protein (HFE) is important in the BMP-SMAD signaling activation. In cases of iron excess, ferritin bound to its transport protein transferrin (Tf-Fe) binds to TFR1, which leads to HFE dissociation from TFR1 and binding to TFR2. D, Iron deficiency, hypoxia, and increased erythropoiesis can downregulate hepcidin through multiple but not completely understood pathways. Under normoxic conditions, prolyl hydroxylases (PHDs) inhibit hypoxia-inducible transcription factors (HIFs). During hypoxia, HIFs upregulate protease matriptase 2 (MT2) that inhibits BMP-SMAD signaling and downregulates hepcidin. Hypoxia can also directly downregulate HAMP transcription. In cases of increased erythropoiesis, erythropoietin (EPO) upregulates erythroferrone (ERFE), which is known to inhibit BMP-SMAD signaling. Other potential erythroid regulators, such as growth and differentiation factor 15 (GDF15), might also downregulate hepcidin.

In this study, we explored the relationship between hepcidin, iron indices, and markers of inflammation, oxidative stress, and hypoxia in patients with active IBD in order to assess the main determinants of hepcidin levels and its potential as a diagnostic ID biomarker. This experimental approach could help to improve the routine assessment and choice of therapy for absolute ID with or without anemia in patients with active IBD.

Methods

Study Population

In this retrospective study, patients with an established IBD diagnosis—Crohn's disease (CD), ulcerative colitis (UC), or IBD unclassified (IBD-U)—were included from a previously described cohort: the Dutch IBD biobank, which prospectively collects medical data and biomaterials for future research.[15] Between January and November 2021, we screened all adult patients included in the IBD biobank at the University Medical Center Groningen (UMCG). Patients who underwent induction therapy with infliximab (IFX) or vedolizumab (VEDO) for clinically, biochemically, or endoscopically active disease were included in this retrospective study. Patients with an indication for biological therapy other than active IBD and patients whose serum samples were available only at week 6 of induction therapy were excluded from the study. In addition, we excluded patients with the following documented comorbidities associated with anemia or abnormalities in hepcidin levels: heart failure, liver cirrhosis, chronic obstructive pulmonary disease, hemoglobinopathies, autoimmune hemolytic anemia, myelodysplastic syndrome, end-stage renal disease (defined by an estimated glomerular filtration rate <30 mL/min/1.73 m^2), or active malignancy except for dermatological nonmelanoma malignancies. Pregnant or lactating women and patients with active infection or documented major surgery (eg, laparoscopic colectomy) within 6 weeks before induction therapy were also excluded.

Moreover, we collected data and serum samples from age- and sex-matched healthy control subjects from a UMCG biobank containing predonation samples of living kidney donors. All data and biomaterials were collected after participants gave written informed consent. The study was approved by the Institutional Review Board at UMCG for patients with IBD (Institutional Review Board no. 2008/338) and for age- and sex-matched healthy control subjects (Institutional Review Board no. 2008/279). The study has been performed in accordance with the principles of the Declaration of Helsinki (2013).

Data Collection

Demographic, anthropometric, and extensive clinical data (**Table 1**) were extracted from medical records. These data were documented at the start of induction therapy. Biochemical parameters were collected at baseline, week 6, and week 14 of induction therapy with either IFX or VEDO. The following biochemical measurements were extracted from medical records: hemoglobin, mean corpuscular volume, white blood cell count (WBC), neutrophils, platelets, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactate dehydrogenase, albumin, creatinine, estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration), serum iron, ferritin, transferrin, total iron binding capacity (TIBC), transferrin saturation (TSAT), and fecal calprotectin (fCal). Montreal classification at baseline and data on clinical, biochemical, and endoscopic or radiologic disease activity were extracted from medical records.[16,17]

Study Outcomes and Definitions

Anemia was defined as hemoglobin <7.5 mmol/L (<120.9 g/L) for women and <8.5 mmol/L (<137.0 g/L) for men based on the Dutch national reference range.[18] ID was defined as ferritin <30 µg/L for healthy control subjects; ID in patients with IBD was defined as ferritin <100 µg/L at baseline, given that all patients had active disease.[4] IDA was defined as concurrent anemia and ID. Functional ID with anemia (ACD) was defined as anemia in combination with ferritin >100 µg/L and TSAT <20%.[4]

Disease activity was evaluated at baseline and the end of the induction therapy (ie, week 14). Clinical disease activity was evaluated using the Simple Clinical Colitis Activity Index for patients with UC or IBD-U[19] and the Harvey-Bradshaw Index for patients with CD.[20] Biochemical disease activity was evaluated by either fCal or CRP measurements (defined as fCal >150 mg/kg and/or CRP >5 mg/L). Endoscopic disease activity was assessed using the Mayo endoscopic subscore for UC and IBD-U; the Simple Endoscopic Score for Crohn's Disease (SES-CD) was used for CD.[21,22] Due to the retrospective nature of the study, endoscopic data were retrieved from medical records at baseline (no more than 90 days before the start of therapy) and after the induction therapy (but no more than 90 days after week 14 of therapy). Endoscopic scores were retrieved from the endoscopy reports or assessed by 2 clinical researchers based on available endoscopic images and endoscopy reports written by certified gastroenterologists employed at UMCG. For patients who underwent capsule endoscopy, disease activity was not evaluated using Mayo or SES-CD scores but was noted as the presence or absence of endoscopic disease activity. Data on available radiologic disease activity were extracted from medical records; IBD disease activity was evaluated by certified radiologists employed at the UMCG who were not involved in the study.

Responders to induction therapy with IFX or VEDO were considered to have an adequate clinical response at week 14 based on normalization or at least a 3-point decrease in Harvey-Bradshaw Index or Simple Clinical Colitis Activity Index scores, or based on the Physician Global Assessment extracted from medical records that reflected any endoscopic, radiologic, biochemical, or clinical improvement. If the Physician Global Assessment was not explicitly mentioned in the medical records, 2 clinical researchers classified the response based on improvement in clinical scores (normalization or at least a 3-point decrease), biochemical improvement (fCal normalization or at least 40% decrease, and/or normalization in CRP), or endoscopic improvement (at least 1-point decrease in endoscopic score). Patients were classified as nonresponders if they did not meet the response criteria or if the induction therapy was ceased before week 14 except for withdrawal due to side effects.

Serum Sample Analysis

Serum samples taken at baseline and week 6 of the induction therapy were stored at -80 °C until analysis. The

Table 1. Baseline demographic and clinical characteristics of the study population

	Control Subjects (n = 50)	CD (n = 66)	UC (n = 56)	Difference (CD and UC)
Female	24 (48%)	40 (60.6%)	18 (32.1%)	<.01[a]
Age, y	45.00 (38.75-51.25)	37.37 (25.58-47.49)	47.00 (33.95-57.10)	<.01[a]
BMI, kg/m^2	26.30 (23.95-28.85)	24.70 (21.70-29.76)	25.40 (22.75-29.84)	.39
Disease duration, y	—	8.88 (5.04-18.00)	8.17 (3.65-14.43)	.29
Montreal classification at baseline				
Age at diagnosis (Montreal A)	—	—	—	<.01[a]
<17 y	—	12 (18.2%)	3 (5.4%)	
17-40 y	—	44 (66.7%)	33 (58.9%)	
>40 y	—	10 (15.2%)	20 (35.7%)	
Disease location (Montreal L)	—	—	—	
Terminal ileum	—	24 (36.4%)	—	
Colon	—	9 (13%)	—	
Ileocolonic	—	33 (50.0%)	—	
Upper GI tract involvement	—	5 (7.6%)	—	
Disease behavior (Montreal B)	—		—	
Inflammatory	—	35 (53.0%)	—	
Stricturing	—	18 (27.3%)	—	
Penetrating	—	13 (19.7%)	—	
Perianal involvement (Montreal p)	—	14 (21.2%)	—	
Disease extension (Montreal E)	—	—	—	
Proctitis	—	—	4 (6.7%)	
Left-sided colitis	—	—	21 (38.2%)	
Pancolitis	—	—	30 (54.5%)	
Surgical and medical history				
Colonic resection[b]	—	5 (7.6%)	3 (5.4%)	.62
Ileocecal resection	—	17 (25.8%)	0 (0%)	
eGFR <60 mL/min/1.73 m^2	0 (0%)	2 (3.1%)	3 (5.5%)	.53
Smoking	—	—	—	<.05
Never	—	17 (28.3%)	20 (39.2%)	
Ex-smoker	—	22 (36.7%)	25 (49.0%)	
Current smoker	—	21 (35.0%)	6 (11.8%)	
Therapy at baseline	—	—	—	
Naive to biologicals	—	39 (59.1%)	28 (50.0%)	.31
Induction with IFX	—	54 (81.8%)	17 (30.4%)	
Induction with VEDO	—	12 (18.2%)	39 (69.6%)	
Aminosalicylates	—	4 (6.1%)	39 (69.6%)	<.001[a]
Immunomodulators	—	—	—	.31
None	—	21 (31.8%)	21 (37.5%)	
Thiopurines	—	40 (60.6%)	34 (60.7%)	
Methotrexate	—	5 (7.6%)	1 (1.8%)	
Local or systemic steroids[c]	—	21 (31.8%)	37 (66.1%)	<.001[a]
Proton pump inhibitors	—	12 (18.2%)	26 (46.4%)	<.001[a]
Iron therapy before induction[d]	—	1 (1.5%)	4 (7.1%)	.12
Disease activity	—	—	—	
HBI score	—	4.00 (2.00-10.00)	—	
SCCAI score	—	—	7.00 (3.00-8.00)	
CRP, mg/L	—	5.20 (1.75-12.50)	2.80 (1.13-5.00)	<.05
fCal, mg/kg	—	667.00 (273.25-2290.00)	830.00 (255.00-1650.00)	.91
Radiologic disease activity	—	16 (100%)[e]	—	
Endoscopic disease activity[f]	—	26 (96.3%)[g]	38 (100%)[h]	.23
Endoscopic Mayo score	—	—	—	
Mayo 1	—	—	4 (10.8%)	

Table 1. Continued

	Control Subjects (n = 50)	CD (n = 66)	UC (n = 56)	Difference (CD and UC)
Mayo 2	—	—	13 (35.1%)	
Mayo 3	—	—	20 (54.1%)	
Endoscopic SES-CD score	—	—	—	
Mild	—	13 (59.1%)	—	
Moderate	—	6 (27.3%)	—	
Severe	—	3 (13.6%)	—	
Anemia and iron deficiency				
Anemia of all causes	4 (8.0%)	31 (47.7%)	21 (37.5%)	.26
Iron deficiency	7 (14.6%)	52 (78.8%)	43 (76.8%)	.79
Iron-deficiency anemia	2 (4.0%)	25 (37.9%)	16 (28.6%)	.28
Anemia of chronic disease	—	5 (7.6%)	3 (5.4%)	.62

Values are n (%) or median (interquartile range). Two-tailed *P* values <.05 were considered statistically significant; the Benjamini-Hochberg procedure was used to adjust for multiple testing, adopting a 5% false discovery rate.
Abbreviations: CD, Crohn's disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; fCal, fecal calprotectin; HBI, Harvey-Bradshaw Index; IBD, inflammatory bowel disease; SCCAI, Simple Clinical Colitis Activity Index; SES-CD, Simple Endoscopic Score for Crohn's Disease; UC, ulcerative colitis.
[a]Statistical significance after adjustment for multiple testing.
[b]History of (partial) colonic resections.
[c]Local enemas or systemic steroids exclusively for IBD treatment.
[d]Within 8 weeks of baseline.
[e]In total, 16 patients had radiologic imaging for disease activity.
[f]Endoscopic disease activity indicates the presence of any endoscopic activity noted during endoscopic procedures regardless of Mayo or SES-CD scores.
[g]In total, 27 patients with CD had endoscopic imaging.
[h]In total, 38 patients with UC/IBD unclassified had endoscopic imaging.

following parameters were measured: hepcidin, free thiols (R-SH, sulfhydryl groups), interleukin (IL)-1β, IL-6, IL-10, IL-22, IL-23, tumor necrosis factor α (TNFα), interferon γ, erythropoietin (EPO), macrophage inflammatory protein 3α (MIP-3α), vascular endothelial growth factor A (VEGF-A), intact fibroblast growth factor 23 (iFGF-23), c-terminal FGF-23 (cFGF-23), and soluble transferrin receptor (sTfR).

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control, or molecular damage.[23] Oxidative stress is associated with biochemical and endoscopic inflammation in patients with IBD.[24] In addition, an overproduction of reactive oxygen species (ie, increased oxidative stress) leads to a reduction in systemic free thiols, which can be quantified in serum. In this study, free thiols (R-SH, sulfhydryl groups) were measured using a colorimetric method described by Bourgonje et al.[24]

Hypoxia and increased erythropoiesis downregulate hepcidin levels.[6] Custom multiplex electrochemiluminescence assays were used to quantify EPO, MIP-3α, and VEGF-A as hypoxia and erythropoiesis-associated markers.[25] In addition, enzyme-linked immunosorbent assays were used to measure cFGF-23 and iFGF-23 because increased cFGF-23 has been associated with erythropoiesis, ID, and acute inflammation, whereas increased iFGF-23 has been associated with chronic inflammation.[26]

Enzyme-linked immunosorbent assays were used to measure hepcidin (R&D Systems; DHP250), sTfR (BioVendor; RD194011100), iFGF-23 (Biomedica; BI-20700), cFGF-23 (Biomedica; BI-20702). Custom electrochemiluminescence assays (Meso Scale Discovery; Meso Scale Diagnostics) were used to measure IL-1β, IL-6, IL-10, IL-22, IL-23, TNFα, interferon γ, EPO, VEGF-A, and MIP-3α. All assays were performed following the manufacturer's instructions after a pilot run to evaluate the appropriate serum sample dilutions.

Statistical Analysis

Descriptive data are reported as mean ± SD or as median (interquartile range [IQR]) for continuous variables. Categorical variables are presented as the proportion and percentage. Normality assessment was performed by visual inspection of normal probability plots (Q-Q) and histograms. Paired analyses were performed using paired *t* tests or Wilcoxon's signed rank tests to evaluate differences between the 2 time points. To compare biochemical parameters and changes (Δ) in parameters between independent (sub)groups, we used Kruskal-Wallis tests, Mann-Whitney *U* tests, chi-square, or independent-sample *t* tests as appropriate. Spearman's rank correlation coefficients (ρ) were used to determine correlations between variables.

Furthermore, univariable and multivariable binary logistic regression analyses were performed to identify parameters that were independently associated with ID or IDA. Multivariable analyses were performed using backward selection ($P_{out} > .05$), with the inclusion of all significantly ($P < .05$) associated variables from the univariable analyses. Receiver-operating characteristic (ROC) curve analysis with the area under the curve (AUC) as an overall measure of fit was used to assess the discriminative capacity of different biochemical parameters to assess ID. ROC curves and corresponding AUCs were calculated using the nonparametric, tie-corrected trapezoidal approximation method.

Two-tailed *P* values <.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% (.05). This approach allowed us to reduce the number of false discoveries derived from multiple hypothesis testing. Statistical analysis was performed with SPSS Statistics 25 software package (IBM) and the Python programming language (v.3.8.6; Python Software Foundation) using the *pandas* (v.1.2.3), *numpy* (v.1.20.0), and

sklearn (v.0.24.2) modules. Data visualization was performed using the *seaborn* (v.0.11.1) and *matplotlib* (v.3.4.1) modules. Missing data and biomarker detection rates are presented in **Supplementary Tables 1 and 2**.

Results

Study Population Characteristics

A total of 130 adult patients were included in the study; however, 8 patients received oral or intravenous iron during induction therapy and were excluded from statistical analyses due to the potential confounding effect on hepcidin levels.[27] In total, 122 patients with IBD (CD: n = 66, UC: n = 48, IBD-U: n = 8) and 50 age- and sex-matched control subjects were analyzed. Patients with IBD were analyzed by stratifying them into 2 groups: CD and UC groups, the latter including patients with UC and IBD-U. **Table 1** presents the baseline demographic and clinical characteristics of the study population. Patients with UC were primarily men (67.9%), significantly older, and diagnosed with UC later in life than patients with CD ($P < .05$). The median disease duration was 8.4 years, which did not differ between the 2 groups. At baseline, 43% of patients with IBD had anemia and 78% of patients had ID. The prevalence of both conditions did not differ between the CD and UC groups.

Compared with healthy control subjects, patients with IBD had lower hemoglobin ($P < .01$) and lower iron indices: ferritin and sTfR ($P < .001$ for both); EPO did not differ between the 2 groups ($P = .06$). As expected, patients with IBD had higher inflammatory parameters such as CRP ($P < .001$), WBC ($P < .01$), and IL-1β and IL-6 ($P < .001$ for both parameters). While inflammation- and hypoxia-associated parameters differed markedly between healthy control subjects and the IBD group, a 36% difference between median hepcidin levels did not reach statistical significance (21.19 ng/mL vs 13.52 ng/mL in control subjects and patients with IBD, respectively; $P = .14$). The differences in the baseline biochemical profile between healthy control subjects and patients with IBD are presented in **Supplementary Table 3**. Comparing patients with CD and UC, the CD group showed a more prominent inflammatory profile with higher CRP (5.20 [95% confidence interval (CI), 1.75-12.50] mg/L vs 2.80 [95% CI, 1.13-5.00] mg/L) for patients with CD and UC, respectively; $P < .05$), platelet count (327.00 [95% CI, 255.50-389.50] × 10^9/L vs 289.00 [95% CI, 239.50-350.25] × 10^9/L for patients with CD and UC, respectively; $P < .05$), and IL-1β concentrations (1.22 [95% CI, 0.95-1.33] pg/mL vs 0.80 [95% CI, 0.13-1.23] pg/mL for patients with CD and UC, respectively; $P < .05$), but these differences were not statistically significant when adjusted for multiple testing (**Supplementary Table 3**). Despite comparable iron indices, a difference in baseline median hepcidin was observed—20.87 ng/mL (CD) vs 10.30 ng/mL (UC)—that was not statistically significant after adjustment for multiple testing (**Supplementary Table 3**).

The Relationship Between Hepcidin and its Regulators: Iron Indices, Inflammation, Oxidative Stress, and Hypoxia

A hierarchically clustered correlation matrix (Figure 2) presents the baseline associations between hepcidin and other biochemical parameters. Hepcidin correlated significantly with 2 iron indices: ferritin (ρ = 0.74) and sTfR/log ferritin Index (ρ = -0.79) ($P < .001$ for both). These associations were also found after 6 weeks of induction therapy and were similar in responders and nonresponders: responders (hepcidin and ferritin: ρ = 0.78; hepcidin and sTfR/log ferritin index: ρ = -0.76) and nonresponders (hepcidin and ferritin: ρ = 0.80; hepcidin and sTfR/log ferritin index: ρ = -0.78). On the other hand, transferrin, serum iron, and sTfR at baseline displayed considerably weaker correlations, while hemoglobin (ρ = -0.01) and TSAT appeared to have little to no association (ρ = -0.12). Compared with iron indices, inflammatory parameters displayed weaker associations with hepcidin. The most prominent correlations—albeit nonsignificant—were between hepcidin and CRP (ρ = 0.50), ESR (ρ = 0.31), and free thiols (ρ = 0.52). Hypoxia-associated serum markers—EPO, MIP-3α, and VEGF-A—also displayed little to no correlation with hepcidin levels. In short, hepcidin levels are predominantly associated with systemic iron status, rather than with inflammation or hypoxia.

ID Determines Hepcidin Levels in an Inflammatory State

We stratified patients based on hepcidin levels at baseline by dividing them into 4 quartiles (**Table 2**). We observed patterns that confirmed the findings presented in the correlation matrix. Patients with hepcidin levels below the median showed at least 93.5% prevalence of ID that decreased to 33.3% in the fourth quartile, representing patients with the highest hepcidin levels. In contrast, ACD was only observed in the fourth quartile (Figure 3). It should be noted that inflammatory parameters such as fCal, ESR, WBC, or TNFα did not show a clear association with hepcidin levels. On the other hand, free thiol levels, which reflect systemic oxidative stress, showed a gradual but nonsignificant increase from the lowest to the highest hepcidin quartile (**Table 2**). To summarize, absolute ID is the primary determinant of lower hepcidin levels even in an inflammatory state, but inflammation without absolute ID increases hepcidin levels.

The associations identified in the correlation matrix were also observed in multivariable analysis. A twofold increase in hepcidin levels showed an odds ratio of 0.24 (95% CI, 0.13-0.43; $P < .001$) for ID (**Supplementary Tables 4 and 5**). In addition, ROC analysis confirmed the relationship between hepcidin and ferritin. At baseline, hepcidin showed a considerable discriminative capacity to differentiate between patients with and without absolute ID: $AUC_{(hepcidin)} = 0.89$ (95% CI, 0.82-0.95; $P < .001$) for the whole IBD cohort. The discriminative capacity was more pronounced in patients with CD (AUC = 0.95) than in patients with UC (AUC = 0.87) (**Supplementary Figure 1**).

Differences Between Responders and Nonresponders to Induction Therapy

Table 3 presents changes in systemic iron status, inflammation, oxidative stress, and hypoxia-associated serum markers throughout induction therapy. At week 6 of induction therapy, we observed 3 main changes regarding systemic iron indices: a decrease in ferritin ($P < .05$), a decrease in hepcidin ($P < .001$), and an increase in transferrin/TIBC ($P < .001$). Routinely used

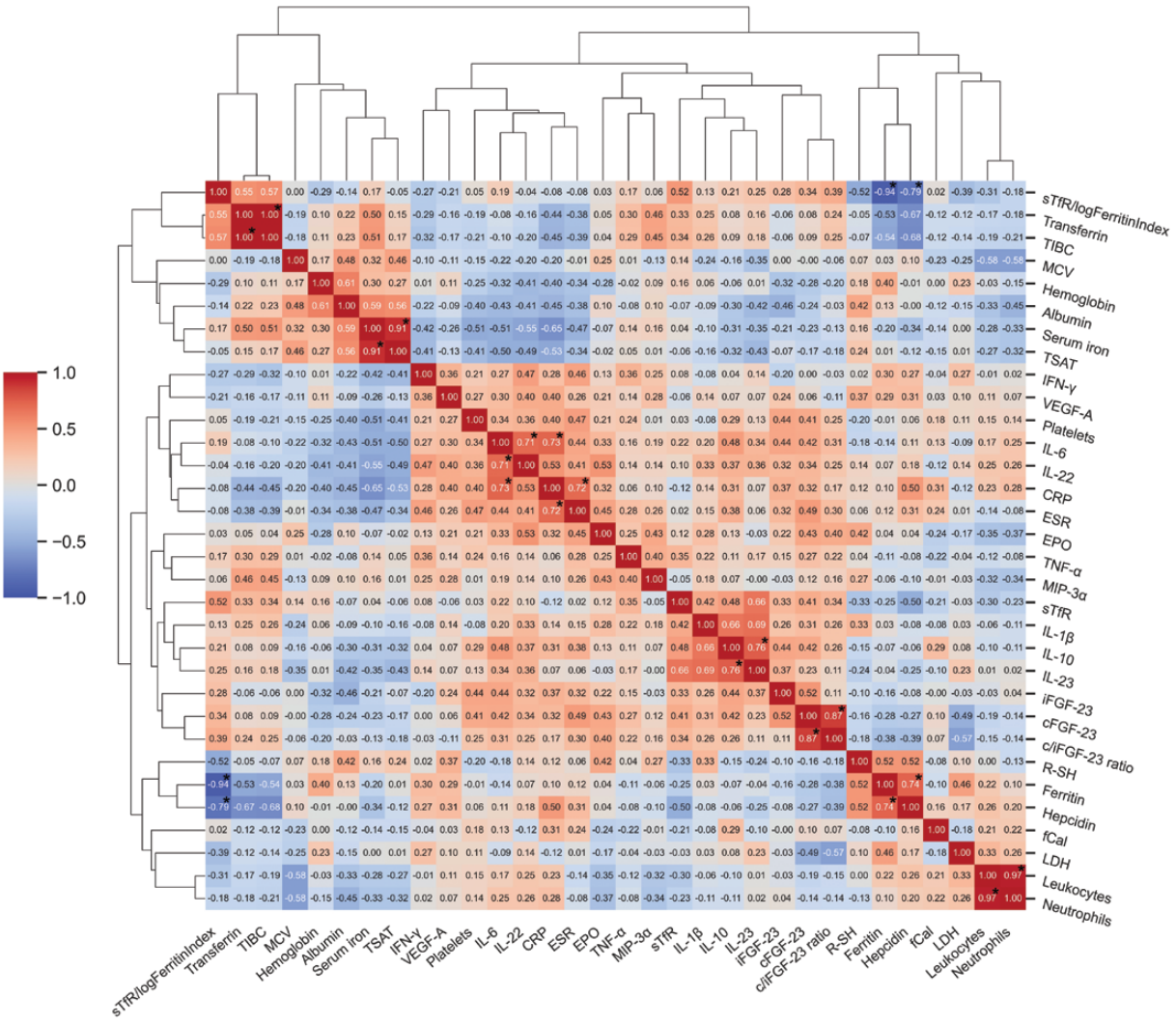

Figure 2. Hierarchically clustered heatmap detailing correlations between different biochemical parameters in patients with active inflammatory bowel disease. The asterisk highlights statistically significant correlations after adjusting for multiple testing. cFGF-23, c-terminal fibroblast growth factor 23; CRP, C-reactive protein; EPO, erythropoietin; ESR, erythrocyte sedimentation rate; fCal, fecal calprotectin; iFGF-23, intact fibroblast growth factor 23; IFN-γ, interferon γ; IL, interleukin; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; MIP-3α, macrophage inflammatory protein 3α; NA, too few data points for statistical testing; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; TNFα, tumor necrosis factor α; TSAT, transferrin saturation; VEGF-A, vascular endothelial growth factor A; WBC, white blood cell count.

inflammatory biomarkers—that is, fCal, CRP, ESR, WBC, neutrophils, and platelets—decreased significantly during the induction therapy. In addition, decreases in hepcidin and inflammation- and hypoxia-associated markers were more evident in patients undergoing IFX therapy compared with VEDO therapy (**Supplementary Table 9**). In short, improvement in inflammatory biomarkers indicated that the increase in transferrin/TIBC and decrease in ferritin and hepcidin, which are positive and negative acute-phase reactants, reflect improvements in inflammation instead of a decline in iron status.

Furthermore, the 3 main changes were observed in patients who responded to the therapy. The differences between patients who responded to induction therapy and those who did not are listed in **Table 4**. Significant improvements were observed in VEGF-A, MIP-3α, and the following inflammatory parameters: fCal CRP, WBC, neutrophils, platelets, IL-6, IL-22, and TNFα. In contrast, no evident changes between baseline and week 6 were observed in patients who did not respond to the therapy (**Table 4**). Last, the differences between responders and nonresponders were also evident when stratifying by the type of biological therapy (**Supplementary Figure 2**). **Supplementary Tables 20 to 24** present changes and differences between patients who responded to the therapy and those who did not, stratified by biological or IBD type. Collectively, these data show that response to induction therapy rather than the type of biological induces changes in hepcidin, iron indices, and inflammation.

Discussion

In this study, we found that hepcidin correlated significantly with iron indices but to a lesser extent with biomarkers of inflammation, oxidative stress, or hypoxia. Even in active IBD, hepcidin differentiated accurately between patients with and without absolute ID. At baseline, hepcidin was generally low

Table 2. Differences in the biochemical profile of patients with inflammatory bowel disease stratified by baseline hepcidin levels, divided into 4 quartiles

	Hepcidin Quartile 1 (n = 30)	Hepcidin Quartile 2 (n = 31)	Hepcidin Quartile 3 (n = 31)	Hepcidin Quartile 4 (n = 30)	
Anemia	13 (43.3%)	15 (48.4%)	10 (32.3%)	14 (48.3%)	.54
Iron deficiency	30 (100%)	29 (93.5%)	26 (83.9%)	10 (33.3%)	<.001[a]
IDA	13 (43.3%)	14 (45.2%)	9 (29.0%)	5 (16.7%)	.06
ACD	0 (0%)	0 (0%)	0 (0%)	8 (26.7%)	<.001[a]
Age, y	40.80 (27.62-49.45)	38.71 (25.95-55.00)	42.00 (28.00-50.00)	46.00 (31.89-59.89)	.53
BMI, kg/m^2	25.34 (22.71-28.31)	24.00 (21.75-31.50)	26.64 (22.20-30.10)	25.00 (21.46-29.79)	.82
Disease duration, y	7.43 (4.30-11.81)	9.61 (3.69-17.41)	9.96 (3.85-18.70)	8.96 (5.44-16.41)	.69
Hemoglobin, mmol/L	8.15 (7.50-8.85)	7.80 (7.30-8.40)	8.10 (7.50-8.90)	8.10 (7.65-8.45)	.71
MCV, fL	87.25 (84.40-92.08)	91.50 (87.60-94.20)	91.65 (86.88-94.00)	90.50 (84.95-93.55)	.15
Iron, µmol/L	11.15 (8.23-14.43)	14.00 (10.00-21.00)	12.75 (8.88-18.33)	11.95 (8.00-16.75)	.36
Ferritin, µg/L	22.50 (15.00-31.75)	33.00 (21.00-46.00)	61.00 (40.00-85.00)	113.00 (74.25-176.00)	<.001[a]
TIBC, µmol/L	71.00 (61.00-85.50)	61.00 (57.00-66.00)	63.50 (56.00-70.00)	54.50 (47.75-59.00)	<.001[a]
Transferrin, g/L	2.80 (2.40-3.43)	2.40 (2.28-2.60)	2.50 (2.20-2.83)	2.20 (1.90-2.40)	<.001[a]
TSAT, %	16.00 (11.00-20.75)	24.00 (13.00-33.00)	19.00 (12.00-28.50)	21.00 (14.75-31.00)	.09
sTfR, µg/mL	9.61 (6.03-11.70)	7.91 (5.98-10.40)	6.54 (5.51-7.93)	7.44 (5.66-8.50)	<.01[a]
sTfR/log ferritin index	6.73 (4.93-9.35)	5.31 (4.16-7.57)	3.64 (2.76-4.84)	3.69 (2.84-4.55)	<.001[a]
CRP, mg/L	3.00 (1.30-4.93)	4.00 (1.60-11.00)	2.65 (1.50-12.75)	9.80 (1.83-27.25)	.06
fCal, mg/kg	740.00 (235.00-1268.00)	1130.00 (360.00-2110.00)	709.00 (255.00-2220.00)	715.00 (183.75-2015.00)	.83
ESR, mm/h	15.00 (9.00-22.00)	23.50 (8.75-40.00)	17.50 (6.00-37.00)	22.00 (6.75-48.00)	.48
WBC, ×10^9/L	7.45 (5.70-8.93)	7.10 (5.40-9.10)	8.00 (6.10-10.30)	8.10 (6.40-10.83)	.38
Neutrophils, ×10^9/L	5.15 (3.78-7.32)	4.50 (3.52-7.57)	5.15 (3.94-7.83)	5.94 (4.31-7.81)	.58
Platelets, ×10^9/L	307.00 (255.75-365.50)	292.00 (260.00-358.00)	329.00 (245.50-380.25)	303.00 (241.00-388.00)	.94
eGFR, mL/min/1.73 m^2	102.00 (91.00-120.25)	111.00 (92.00-119.00)	102.00 (80.00-117.75)	98.00 (86.00-115.75)	.45
Albumin, g/L	42.00 (39.50-43.00)	42.00 (39.00-43.00)	41.00 (40.00-42.25)	40.50 (39.00-44.25)	1.00
LDH, U/L	173.00 (143.50—203.50)	157.00 (135.00-222.50)	170.50 (140.50-213.75)	175.50 (149.75-205.25)	.84
cFGF-23, pmol/L	1.33 (0.81-2.89)	0.74 (0.41-1.59)	0.56 (0.26-0.93)	0.99 (0.73-1.32)	<.01[a]
iFGF-23, pg/mL	10.12 ± 4.95	9.33 (7.13-13.14)	9.80 (7.30-11.61)	11.96 (6.38-16.75)	.62
c/iFGF-23 ratio	0.14 (0.05-0.28)	0.10 (0.05-0.16)	0.06 (0.03-0.10)	0.07 (0.05-0.13)	.02
IL-1β, pg/mL	1.17 (0.87-1.44)	1.12 (0.63-1.27)	0.90 (0.06-1.23)	1.14 (0.28-1.35)	.29
IL-6, pg/mL	2.15 (0.94-3.79)	1.93 (0.96-3.44)	2.46 (0.92-3.75)	2.78 (1.25-5.22)	.65
IL-10, pg/mL	1.43 (0.67-1.59)	1.07 (0.50-1.56)	0.59 (0.48-1.36)	1.23 (0.39-1.59)	.15
IL-22, pg/mL	1.17 (0.67-1.43)	1.21 (0.71-2.42)	1.07 (0.84-2.75)	1.24 (0.87-2.89)	.77
IL-23, pg/mL	7.67 (1.23-9.34)	6.76 (3.38-7.56)	6.58 (0.57-7.86)	6.57 (0.87-7.84)	.53
TNFα, pg/mL	2.21 (1.56-2.95)	2.07 (1.53-2.58)	2.07 (1.62-3.11)	1.93 (1.46-3.20)	.81
IFN-γ, pg/mL	18.19 (11.98-31.11)	10.87 (5.77-30.04)	15.00 (7.88-29.73)	25.02 (12.33-54.24)	.15
EPO, pg/mL	84.63 (51.37-150.11)	100.77 (70.49-148.64)	73.83 (45.19-89.33)	78.06 (50.87-122.70)	.11
MIP-3α, pg/mL	21.66 (12.79-34.04)	16.70 (10.65-29.77)	20.92 (10.66-38.87)	19.51 (12.32-25.39)	.73
VEGF-A, pg/mL	153.20 (84.98-274.10)	86.06 (56.22-120.09)	120.56 (88.17-193.08)	158.35 (83.65-295.05)	.02
R-SH, µM	220.18 (185.39-267.94)	231.28 (191.31-246.91)	237.23 (206.07-275.56)	257.84 (208.25-279.35)	.48

Values are n (%) or median (interquartile range). Hepcidin quartile 1 includes hepcidin levels under 4853.325 pg/mL, quartile 2 includes hepcidin levels between 4853.325 and 13 519.515 pg/mL, quartile 3 includes values between 13 519.5151 and 28 718.1825 pg/mL, and quartile 4 includes hepcidin values >28 718.1825 pg/mL. Two-tailed *P* values <.05 were considered statistically significant; the Benjamini-Hochberg procedure was used to adjust for multiple testing, adopting a 5% false discovery rate.

Abbreviations: ACD, anemia of chronic disease; cFGF-23, c-terminal fibroblast growth factor 23; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; ESR, erythrocyte sedimentation rate; fCal, fecal calprotectin; IDA, iron deficiency anemia; iFGF-23, intact fibroblast growth factor 23; IFN-γ, interferon γ; IL, interleukin; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; MIP-3α, macrophage inflammatory protein 3α; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; TNFα, tumor necrosis factor α; TSAT, transferrin saturation; VEGF-A, vascular endothelial growth factor A; WBC, white blood cell count;

[a]Statistical significance after adjustment for multiple testing.

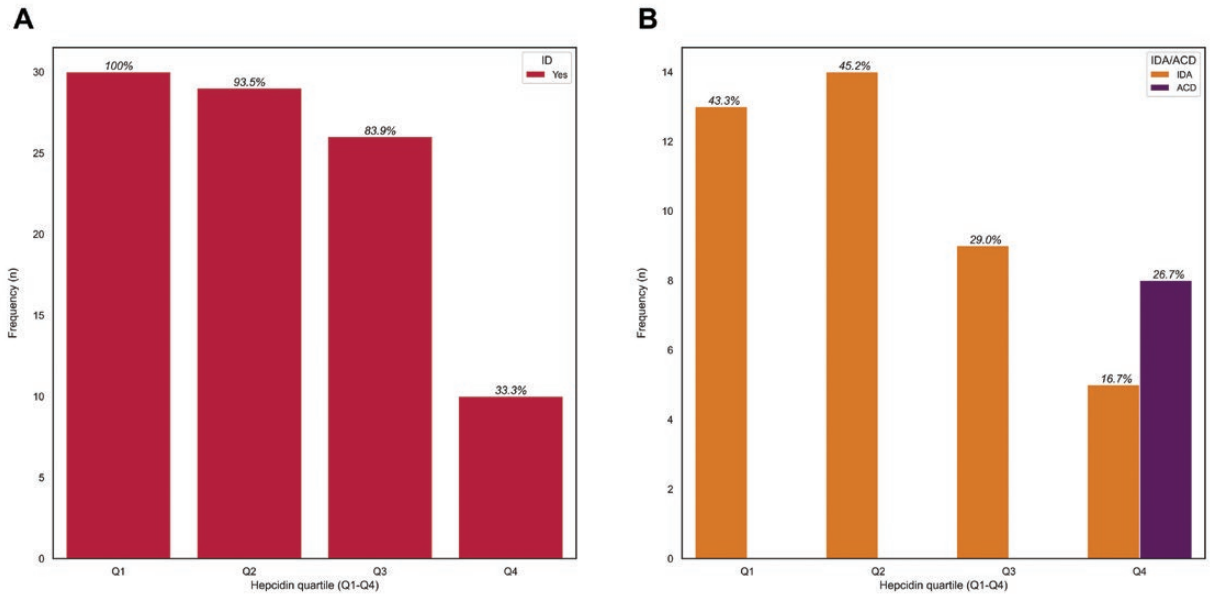

Figure 3. The prevalence of iron deficiency (ID), iron deficiency anemia (IDA), and anemia of chronic disease (ACD) in patients with inflammatory bowel disease, stratified by baseline hepcidin levels divided into quartiles. Hepcidin quartile 1 (Q1) includes hepcidin levels under 4853.325 pg/mL, Q2 includes hepcidin levels between 4853.325 and 13 519.515 pg/mL, Q3 includes values between 13 519.5151 and 28 718.1825 pg/mL, and Q4 includes hepcidin values >28 718.1825 pg/mL.

in patients with concurrent ID and inflammation, whereas hepcidin levels were high in patients with inflammation but without ID. In addition, we found that induction therapy with either IFX or VEDO increased transferrin and TIBC but reduced hepcidin, ferritin, and inflammatory parameters at week 6, which is in line with existing evidence regarding changes in iron parameters during anti-inflammatory treatment. These effects were the most prominent in patients responding to induction therapy. Collectively, these findings indicate that inflammation can affect hepcidin levels; however, absolute ID is the primary determinant of low hepcidin levels, even in an inflammatory state. Hence, hepcidin could be a potential biomarker for absolute ID in patients with active IBD and could aid in therapeutic decision making, given that elevated hepcidin levels are associated with enteral iron malabsorption.

Inflammation and ID—opposing hepcidin-regulating stimuli—coexist in patients with IBD and make it challenging to determine which regulatory stimuli are the main determinants of hepcidin levels (Figure 1). In this study, we found that systemic iron indices, specifically ferritin and sTfR/log ferritin index, were significantly correlated with hepcidin. These findings are in line with previous studies in which data showed a positive and significant correlation between hepcidin and ferritin in patients with IBD.[7-9,11,13,28-30] In addition, our study shows that lower hepcidin levels are highly indicative of absolute ID, as observed in logistic regression and ROC analyses, and can accurately discriminate between patients with and without ID.

In contrast, reported data on the association between hepcidin and inflammation in patients with IBD are inconsistent. We found an association between hepcidin and oxidative stress, CRP, and ESR, but these associations did not reach statistical significance after adjustment for multiple testing (Figure 2). Surprisingly, we did not see stronger correlations between hepcidin and IL-6 or IL-1β despite their critical role in the JAK2-STAT3 pathway and C/EBPα-signaling (Figure 1). Positive correlations between hepcidin and IL-6 or CRP were observed in several but not all prior studies.[9,10,12,28,30-33] Also, we observed an interesting pattern when analyzing patients divided into quartiles based on hepcidin levels at baseline: oxidative stress was higher in patients with lower hepcidin levels. Oxidative stress has been associated with inflammation in patients with IBD; therefore, we expected to observe increased oxidative stress in patients with high hepcidin rather than low hepcidin levels.[24] Interestingly, (ID) anemia has also been associated with increased oxidative stress.[34] Based on our data, we cannot conclude whether increased oxidative stress in our study population is primarily associated with IBD disease activity or ID. This association should be explored further in patients with and without concurrent inflammation and ID, and any combination thereof.

Our results show little to no correlation between hepcidin and hypoxia or erythropoiesis. At baseline, healthy control subjects and patients with IBD had similar levels of EPO and cFGF23 even though ID and anemia were prevalent in patients with IBD. We postulate that inflammatory cytokines, which were elevated in patients with IBD compared with healthy control subjects, suppressed erythropoiesis markers. The effect of inflammation on erythropoiesis could have affected the (lack of) association we observed between hepcidin and erythropoiesis. To our knowledge, these relationships have not yet been investigated in patients with IBD. Future studies should evaluate patients with and without ID during quiescent IBD to avoid the potential confounding effect of inflammatory cytokines.

In short, we observed that ID determined low hepcidin levels regardless of hypoxia, erythropoiesis, oxidative stress, or inflammation. Similarly, Mecklenburg et al[29] reported that in patients with IBD, who had ferritin levels <30 µg/L, hepcidin levels were low regardless of inflammation.

Table 3. Changes in inflammation and systemic iron parameters during induction therapy with either infliximab or vedolizumab in patients with Inflammatory Bowel Disease.

	Baseline(n = 122)	Week 6 (n = 119)	Week 14 (n = 114)	Δ Baseline to Week 6	Δ Baseline to Week 14
Hemoglobin, mmol/L	8.06 ± 0.86	8.00 (7.60-8.70)	8.10 (7.70-8.70)	<.05[a]	<.01[a]
Women	7.70 (7.30-8.33)	7.70 (7.35-8.20)	7.90 (7.55-8.20)	.57	.08
Men	8.30 (7.80-9.10)	8.50 (8.00-9.20)	8.50 (8.00-8.95)	<.01[a]	.04
MCV, fL	90.45 (85.83-93.38)	90.15 (86.45-94.00)	90.00 (86.50-92.55)	.28	.31
Ferritin, µg/L	45.50 (23.75-92.00)	37.00 (23.00-71.50)	37.00 (23.25-66.75)	<.05[a]	<.001[a]
Iron, µmol/L	12.80 (8.35-17.15)	13.90 (9.50-18.70)	13.60 (8.00-19.00)	.26	.23
Transferrin, g/L	2.40 (2.20-2.80)	2.50 (2.30-2.90)	2.60 (2.30-2.90)	<.001[a]	<.001[a]
TIBC, µmol/L	61.00 (55.50-69.00)	64.00 (57.50-73.00)	64.50 (59.00-73.00)	<.001[a]	<.001[a]
TSAT, %	19.00 (13.00-28.00)	21.00 (13.00-28.00)	21.00 (12.25-30.75)	.73	.77
Hepcidin, ng/mL	13.52 (4.85-28.72)	9.49 (2.83-21.60)	NA	<.001[a]	NA
sTfR, µg/mL	7.39 (5.87-10.06)	7.49 (6.17-9.65)	NA	.45	NA
sTfR/log ferritin index	4.56 (3.42-6.19)	4.88 (3.47-6.61)	NA	<.05[a]	NA
EPO, pg/mL	81.47 (54.32-131.05)	69.26 (54.00-123.15)	NA	.11	NA
MIP-3α, pg/mL	19.82 (11.06-31.14)	16.70 (10.85-26.51)	NA	<.05[a]	NA
VEGF-A, pg/mL	119.61 (77.56-212.68)	112.14 (66.78-188.64)	NA	<.05[a]	NA
R-SH, µM	233.90 (193.49-274.89)	241.86 ± 58.79	NA	.14	NA
ESR, mm/h	18.00 (8.00-38.00)	12.00 (5.50-28.00)	11.00 (6.00-23.00)	<.001[a]	<.001[a]
CRP, mg/L	3.60 (1.60-11.00)	2.00 (0.90-5.00)	2.30 (0.80-7.00)	<.001[a]	<.01[a]
WBC, $\times 10^9$/L	7.75 (5.88-10.13)	6.50 (5.10-7.90)	6.20 (4.90-7.80)	<.001[a]	<.001[a]
Neutrophils, $\times 10^9$/L	5.14 (3.84-7.61)	4.03 (3.01-5.11)	3.82 (2.84-5.32)	<.001[a]	<.001[a]
Platelets, $\times 10^9$/L	304.50 (249.75-370.75)	277.00 (242.00-343.00)	268.50 (238.75-326.25)	<.01[a]	<.01[a]
fCal, mg/kg	750.00 (258.50-1843.50)	NA	150.00 (43.00-850.00)	NA	<.01[a]

Values are mean ± SD or median (interquartile range). Two-tailed *P* values <.05 were considered statistically significant; the Benjamini-Hochberg procedure was used to adjust for multiple testing, adopting a 5% false discovery rate.
Abbreviations: CRP, C-reactive protein; EPO, erythropoietin; fCal, fecal calprotectin; MCV, mean corpuscular volume; MIP-3α, macrophage inflammatory protein 3α; NA: not measured; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; TSAT, transferrin saturation; VEGF-A, vascular endothelial growth factor A; WBC, white blood cell count.
[a]Statistical significance after adjustment for multiple testing.

Comparable findings were reported in healthy women and animal studies.[35-37] Altogether, these data suggest that inflammation without ID increases hepcidin levels, but when ID and inflammation coexist, ID prevails in the regulation of hepcidin. This leads us to believe that hepcidin could be a viable ID biomarker in IBD.

Furthermore, we demonstrate that induction therapy—especially in patients who responded to the therapy—significantly decreased hepcidin, which might lead to better iron bioavailability. When comparing patients with and without absolute ID at baseline, we noticed that after 6 weeks of induction therapy, CRP, WBC, hepcidin, and ferritin levels decreased in patients without absolute ID, but the average hemoglobin and TSAT levels increased (**Supplementary Table 10**). This leads us to believe that decreases in hepcidin levels during induction therapy are related to improvements in inflammation, rather than to a decline in iron status. These findings are in line with previous studies that showed a decrease in hepcidin during anti-inflammatory therapy in patients with IBD. This decrease was significant in patients who responded to the treatment compared with those who did not.[30,32,38,39] Likewise, these trends were observed in other patient populations (eg, in patients with rheumatoid arthritis).[40] Because most iron indices are also acute-phase proteins (ie, inflammation increases ferritin and decreases transferrin), previous data and our findings suggest that appropriate anti-inflammatory treatment during phases of active IBD can reduce hepcidin levels and, consequently, might improve the bioavailability of iron supplements.

This is the first study to focus on changes in hepcidin levels and its regulatory stimuli during induction therapy with 2 different types of biologicals in a well-defined cohort of patients with IBD. However, this study has several limitations. First, data on endoscopic disease activity were not available for all included patients. We had to rely on clinical or biochemical disease activity assessment, which lacked standardized objective substantiation. In addition, at least a third of the study population was pretreated with different doses of local or systemic steroids. Second, due to the retrospective nature of the study, we did not have data on whether patients were receiving iron therapy through their general practitioner or over-the-counter supplements, as well as their dietary intake of iron (ie, whether patients ate a vegetarian or vegan diet). Also, we defined ID at baseline based on ferritin, an acute-phase protein that can be elevated during inflammation, which might have influenced predictive analysis in this study. Third, several measured biomarkers showed concentrations in the lower ranges of the calibration curves (**Supplementary Table 2**) that could be associated with somewhat lower accuracy, but all reported data were obtained from samples within the dynamic range of the assays and above the detection limits of the assays used. Finally, a larger

Table 4. Changes in biochemical parameters in patients with inflammatory bowel disease, stratified by the response at week 14 to induction therapy with either infliximab or vedolizumab

	Responders			Nonresponders			Δ Difference Between Groups
	Baseline (n = 92)	Week 6 (n = 92)	Paired Analysis	Baseline (n = 23)	Week 6 (n = 22)	Paired Analysis	
Hemoglobin, mmol/L	8.13 ± 0.86	8.22 ± 0.90	.06	7.90 (7.20-8.30)	8.00 (7.40-8.50)	.55	.83
Women	7.70 (7.30-8.30)	7.75 (7.30-8.15)	.44	7.60 (7.05-8.38)	7.70 (7.25-8.15)	.60	.45
Men	8.53 ± 0.81	8.60 (8.00-9.30)	<.05[a]	7.90 (7.60-8.30)	8.15 (7.78-8.55)	.23	.91
Systemic iron status parameters							
Ferritin, µg/L	46.00 (27.00-91.50)	38.50 (24.25-71.00)	<.001[a]	29.00 (20.00-114.00)	34.50 (16.50-104.25)	.18	.80
Iron, µmol/L	14.00 (9.00-18.00)	14.20 (10.10-19.00)	.43	8.50 (5.20-13.80)	12.30 (6.38-15.85)	.25	.42
Transferrin, g/L	2.40 (2.20-2.80)	2.50 (2.38-2.90)	<.001[a]	2.40 (2.18-2.70)	2.40 (2.18-3.05)	.23	.35
TIBC, µmol/L	61.00 (56.00-70.00)	64.00 (58.00-73.00)	<.001[a]	61.00 (54.00-69.00)	59.50 (53.75-77.75)	.29	.44
TSAT, %	21.00 (14.00-30.00)	21.00 (14.25-29.00)	.92	18.00 (8.00-22.25)	23.50 (9.50-27.00)	.43	.44
Hepcidin, ng/mL	13.65 (4.87-29.74)	9.09 (3.26-21.14)	<.001[a]	10.18 (4.79-24.22)	12.11 (2.67-24.47)	.29	.40
sTfR, µg/mL	7.52 (5.80-10.09)	7.33 (6.16-9.56)	.86	7.25 (6.00-10.81)	8.16 (5.97-10.60)	.26	.28
sTfR/log Ferritin Index	4.53 (3.47-6.14)	4.77 (3.50-6.33)	.06	4.83 (3.34-8.18)	5.12 (3.40-8.33)	.16	.39
Inflammation-associated parameters							
CRP, mg/L	3.20 (1.30-11.00)	1.75 (0.60-4.60)	<.001[a]	5.00 (2.80-15.00)	5.50 (1.18-13.25)	.09	.92
ESR, mm/h	16.00 (7.00-34.00)	11.00 (4.00-22.00)	<.001[a]	28.00 (15.00-46.00)	29.00 (13.50-37.25)	.35	.30
WBC, $\times 10^9$/L	7.45 (5.70-9.78)	6.35 (4.78-7.88)	<.001[a]	8.20 (6.20-11.10)	7.05 (6.08-9.10)	<.05	.93
Neutrophils, $\times 10^9$/L	5.07 (3.70-7.37)	3.99 (2.85-4.99)	<.01[a]	6.48 (4.20-8.89)	4.75 (3.98-6.42)	<.05	.79
Platelets, $\times 10^9$/L	298.00 (247.50-365.75)	275.00 (242.00-316.00)	<.01[a]	345.50 (254.75-373.00)	313.00 (243.00-398.00)	.69	.15
cFGF-23, pmol/L	0.86 (0.50-1.33)	0.89 (0.44-1.61)	.21	1.08 (0.41-2.67)	1.29 (0.59-1.67)	.86	.71
iFGF-23, pg/mL	9.94 (6.56-13.98)	9.60 (6.09-12.90)	.21	10.37 (8.02-13.00)	8.96 (7.36-12.71)	.32	.49
c/iFGF-23 ratio	0.09 (0.05-0.15)	0.10 (0.05-0.18)	.47	0.08 (0.04-0.21)	0.11 (0.06-0.20)	.53	.37
IL-1β, pg/mL	1.10 (0.23-1.27)	1.05 (0.57-1.30)	.32	1.07 (0.38-1.40)	1.20 (0.85-1.33)	.88	.63
IL-6, pg/mL	2.19 (1.03-3.68)	1.51 (0.99-2.60)	<.01[a]	2.74 (1.05-5.74)	2.66 (1.13-4.13)	1.00	.21
IL-10, pg/mL	1.11 (0.47-1.58)	0.92 (0.47-1.73)	.18	0.96 (0.52-1.53)	0.76 (0.52-1.58)	.13	.53
IL-22, pg/mL	1.15 (0.71-1.78)	0.91 (0.56-1.37)	<.001[a]	1.24 (0.71-2.34)	1.04 (0.86-2.15)	.17	.61
IL-23, pg/mL	6.85 (0.80-7.91)	6.73 (0.90-8.35)	.66	6.88 (1.46-8.06)	7.79 (5.08-8.68)	.77	.99
TNFα, pg/mL	2.03 (1.51-2.57)	1.75 (0.92-2.35)	<.01[a]	2.07 (1.53-3.39)	2.60 (1.15-3.28)	.81	.21
IFN-γ, pg/mL	18.08 (8.72-31.24)	14.76 (7.43-31.80)	.18	15.24 (7.01-30.55)	15.33 (10.74-33.65)	.88	.62
R-SH, µM	233.90 (194.76-274.26)	241.22 (203.90-290.13)	<.05	228.99 (186.12-244.96)	213.35 (174.75-232.52)	.10	<.05
fCal, mg/kg[b]	805.00 (250.00-1756.75)	120.00 (40.50-206.00)	<.001[a]	790.00 (633.75-2725.00)	2030.00 (520.00-2775.00)	1.00	.07
Hypoxia-associated parameters							
EPO, pg/mL	83.92 (51.44-136.19)	67.09 (53.98-121.56)	<.05[a]	70.49 (54.59-99.99)	87.95 (48.00-125.99)	.24	<.05
MIP-3α, pg/mL	18.60 (10.64-31.66)	16.19 (9.31-27.10)	<.05[a]	21.60 (17.81-29.89)	17.77 (12.97-24.66)	.65	.29
VEGF-A, pg/mL	117.84 (73.15-208.75)	113.41 (66.74-182.00)	.08	105.14 (79.36-226.82)	111.54 (54.72-211.75)	.57	.53
Other parameters							
MCV, fL	90.05 (85.78-93.30)	90.30 (86.60-94.10)	.43	90.50 (82.50-92.60)	89.14 ± 5.82	.70	.67
LDH, U/L	173.00 (143.00-205.50)	178.00 (148.00-212.75)	.07	165.50 (141.00-269.00)	168.00 (130.50-261.50)	.56	.24
Albumin, g/L	41.50 (40.00-44.00)	43.00 (41.00-44.00)	<.01[a]	40.00 (38.00-43.00)	41.00 (38.50-42.00)	.64	.10

Values are mean ± SD or median (interquartile range). 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.
Abbreviations: cFGF-23, c-terminal fibroblast growth factor 23; CRP, C-reactive protein; EPO, erythropoietin; ESR, erythrocyte sedimentation rate; fCal, fecal calprotectin; iFGF-23, intact fibroblast growth factor 23; IFN-γ, interferon γ; IL, interleukin; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; MIP-3α, macrophage inflammatory protein 3α; NA, too few data points for statistical testing; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; TNFα, tumor necrosis factor α; TSAT, transferrin saturation; VEGF-A, vascular endothelial growth factor A; WBC, white blood cell count.
[a]Statistical significance after adjustment for multiple testing.
[b]fCal measured before and after the induction therapy.

sample size would have allowed us to establish more reliable subgroup analyses and to adjust for confounding variables more extensively.

Despite the limitations, this study shows that inflammation without ID can increase hepcidin levels, but ID is still the primary determinant of low hepcidin levels, even in an

inflammatory state. In addition, this study provides evidence that treating IBD-associated intestinal inflammation will reduce hepcidin levels and, consequently, might improve enteral iron absorption because elevated hepcidin is associated with enteral iron malabsorption in patients with IBD.[8,13,14] Interestingly, hepcidin has been shown to predict (non)responsiveness to iron therapy in patient populations beyond IBD.[41,42] To this date, all biochemical parameters used in routine care and clinical trials to evaluate iron indices are affected by inflammation or show poor diagnostic value, which complicates the interpretation of iron stores and study findings.[43-46] Hence, prospective studies focusing on iron status based on bone marrow aspirates or oral iron absorption tests are necessary to assess and validate a cutoff value for hepcidin in order to differentiate between ID and functional iron restriction in patients with IBD. In addition, prospective studies should evaluate whether hepcidin can predict response to intravenous or enteral iron therapy that would prove hepcidin useful not only in the diagnosis, but also in the treatment of ID and iron restriction, which could render hepcidin a considerably more valuable biomarker than ferritin.

Conclusions

Inflammation affects hepcidin levels. However, ID is the primary determinant of low hepcidin levels, even in an inflammatory state. Hepcidin accurately differentiates between patients with and without absolute ID. In addition, induction therapy can reduce hepcidin levels, which might lead to better iron bioavailability. Further studies are necessary to optimize the diagnosis and treatment of ID in IBD with emphasis on hepcidin as a diagnostic, or even therapeutic, biomarker.

Supplementary data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Author Contributions

R.L., A.R.B., A.E.v.d.M.-d.J., and G.D. conceptualized and designed the study. G.D. and A.E.M.J. were responsible for funding acquisition and resources. G.D., M.C.V., E.A.M.F., H.M.v.D., and R.K.W. included subjects with IBD. R.L., A.R.B., A.E.v.d.M.-d.J., and G.D. collected study data and materials. J.J.v.d.R., L.J.A.C.H., M.L.C.B., H.v.G., and R.L. supervised and performed biomarker measurements. R.L. and A.R.B. performed data analysis and data visualization. R.L. wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read, and approved the final version of the manuscript to be submitted for publication.

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Conflict of Interest

G.D. has received research grants from the Royal DSM; and speaker's fees from Janssen Pharmaceuticals, Takeda, Pfizer, and AbbVie. A.E.v.d.M.-d.J. has received unrestricted research grants from Galapagos, Norgine, Vedanta, and Nestlé; and speaker fees from Galapagos, Tramedico, Takeda, and Janssen Pharmaceuticals. L.J.A.C.H. has received unrestricted research grants from TRACON pharmaceuticals; and has a patent with co-inventorship. M.C.V. has served on the advisory board for Janssen-Cilag and received a speaker fee from Takeda, outside the submitted work. R.K.W. has served as consultant for Takeda; received unrestricted research grants from Takeda, Johnson & Johnson, Tramedico, and Ferring; and received speaker fees from MSD, AbbVie, and Janssen Pharmaceuticals. R.L. has served on the advisory board and received travel expenses from Cablon Medical. All other authors have no conflicts of interest to declare.

Data Availability

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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