Hepcidin, Interleukin-6 Levels and Iron Metabolism Parameters in Patients with Hepatic Glycogen Storage Diseases: A Cross-Sectional Study

Journal of Inborn Errors
of Metabolism & Screening
2024, Volume 12: e20230015
DOI: https://doi.org/10.1590/2326-4594-JIEMS-2023-0015

Tatiéle Nalin[1,2], Fernanda Sperb-Ludwig[2,3,4], Marina Siebert[4,5,6], Gustavo Mottin Rizowy[2,3], David A. Weinstein[7,8], Terry G. J. Derks[9], Carolina F. M. de Souza[10] and Ida V. D. Schwartz[2,3,4,10,11]

Abstract

Hepatic glycogen storage diseases (GSD) are characterized by recurrent episodes of hypoglycemia, and anemia has been recognized as a frequent complication of these disorders. This was a convenience cross-sectional study to evaluate hepcidin and IL-6 concentrations in patients with hepatic GSD and their association with anemia and other parameters of iron metabolism. Levels of hepcidin, IL-6, and markers of iron metabolism were measured in 32 patients receiving uncooked cornstarch therapy for GSD (GSD Ia= 18; Ib= 7; III= 3; IXa= 3; IXb= 1; median age 9.5 years). IL-6 concentrations were compared to those of 8 individuals heterozygous for GSD. Nine patients were anemic and five patients had hepatic adenomas. IL-6 levels were higher in patients than in heterozygotes. Eight patients had hyperferritinemia, and one had elevated transferrin saturation as well. Hepcidin correlated positively with ferritin levels. IL-6 correlated with hemoglobin, iron, transferrin, and transferrin saturation. There was no correlation between hepcidin and IL-6 levels. Patients with GSD Ib had the highest IL-6 levels. Anemia is a common finding in hepatic GSD, especially in GSD Ib, the type of GSD associated with the highest IL-6 levels. These findings suggest that inflammation is strongly associated with development of anemia in GSD Ib.

Keywords

Glycogen storage diseases, cytokines, anemia, iron metabolism, hepcidin.

Introduction

The hepatic glycogen storage diseases (GSD) are inborn errors of metabolism characterized by abnormal endogenous glucose production and, consequently, fasting hypoglycemia. These conditions are classified into different types and receive different names depending on the underlying gene and enzyme defect and differ in terms of chemical and biochemical manifestations. The most frequent forms presented by patients are type Ia, Ib, III, IXa and IXc (Table 1).

The most common management strategy is frequent administration of uncooked cornstarch, aiming at maintaining normal blood glucose levels and preventing secondary metabolic derangements. Additional dietary modifications may be made depending on the type of GSD [1-10].

[1]Ultragenyx Brasil Farmacêutica Ltda, São Paulo, SP, Brazil.
[2]Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Porto Alegre, RS, Brazil.
[3]Hospital de Clínicas de Porto Alegre, Laboratório de Pesquisa Básica e Investigação Avançada em Neurociências, Porto Alegre, RS, Brazil.
[4]Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Medicina: Ciências Médicas, Porto Alegre, RS, Brazil.
[5]Hospital de Clínicas de Porto Alegre, Unidade de Pesquisa Laboratorial, Centro de Pesquisa Experimental, Porto Alegre, RS, Brazil.
[6]Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências da Gastroenterologia e Hepatologia, Porto Alegre, RS, Brazil.
[7]Connecticut Children's Medical Center, Glycogen Storage Disease Program, Hartford, United States.
[8]University of Connecticut School of Medicine, Department of Pediatrics, Farmington, United States.
[9]University Medical Center of Groningen, Beatrix Children's Hospital, Section of Metabolic Diseases, Groningen, The Netherlands.
[10]Hospital de Clínicas de Porto Alegre, Serviço de Genética Médica, Porto Alegre, RS, Brazil.
[11]Universidade Federal do Rio Grande do Sul, Departamento de Genética, Porto Alegre, RS, Brazil.

Received November 30, 2023. Accepted for publication June 19, 2024

Corresponding Author:
Fernanda Sperb-Ludwig, E-mail: fsperb@hcpa.edu.br

Table 1. Characterization of the most frequent types of hepatic glycogenosis.

GSD type	Gene	OMIM	Location	Enzyme deficiency	Inheritance	Main clinical symptoms		
						Hypoglycemia	Hepatomegaly	Hyperlipidemia
0	*GYS2*	138571	12p12.2	glycogen synthase	AR	Yes	No	No
Ia	*G6PC*	232200	*17q21*	glucose-6-phosphatase	AR	Yes	Yes	Yes
Ib	*SLC37A4*	232220	11q23.3	glucose-6-phosphate transporter	AR	Yes	Yes	Yes
III	*AGL*	232400	1p21	glycogen debranching enzyme	AR	Yes	Yes	Yes
IV	*GBE1*	232500	3p12.3	glycogen branching enzyme	AR	No	Yes	No
VI	*PYGL*	232700	14q21-q22	liver glycogen phosphorylase	AR	Yes	Yes	Yes
XIa	*PHKA2*	306000	Xp22.2-p22.1	phosphorylase kinase α subunit	X-linked	Yes	Yes	No
XIb	*PHKB*	261750	16q12-q13	phosphorylase kinase β subunit	AR	Yes	Yes	No
XIc	*PHKG2*	613027	16p12.1-p11.2	phosphorylase kinase γ subunit	AR	Yes	Yes	Yes
XI	*SLC2A2*	612933	3q26.1-q26.2	facilitated glucose transporter	AR	Yes	Yes	Yes
XII	*ALDOA*	611881	16q22-q24	aldolase A	AR	No	Yes	No

Anemia is a common complication associated with GSD. An European study found a prevalence of hemoglobin values below the reference values for approximately 25% of prepubertal GSD Ia and 50% of prepubertal GSD Ib patients, 40% and 70% for adolescents with GSD Ia and GSD Ib, respectively, and 45% and 100% for adults with GSD Ia and GSD Ib, respectively [11]. In a US study, Talente et al. [12] described the presence of anemia in 26 (81%) of 32 adult patients with GSD Ia. In addition, of the 5 adults patients with GSD Ib studied, all were declared as anemic. A study by Wang et al. [13] from US, described a prevalence of anemia of 41.7% in patients with GSD Ia and 71.8% in patients with GSD Ib. Research on the pathogenesis of anemia, however, has been limited, and all prior studies have been limited to GSD type I. Previous investigations have suggested that different mechanisms may be involved in the pathogenesis of anemia in GSD Ia and Ib. Anemia in GSD Ia is reportedly associated with iron deficiency or hepatic adenomas; in contrast, inflammation related to GSD enterocolitis has been associated with anemia in GSD Ib [13-15]. While different mechanisms have been implicated, hepcidin is linked to both of these processes [16-17]. Hepcidin is a hormone produced by the liver which is a key regulator of iron homeostasis [18-19]. It has been linked to hepatic adenomas in GSD and also anemia of chronic disease [14,16,20].

To better understand the pathogenesis of anemia in GSD, we performed an observational, prospective, cross-sectional study of plasma concentrations of hepcidin and interleukin-6 and iron homeostasis parameters in a single-center GSD cohort.

Materials and Methods

The study was approved by the Hospital de Clínicas de Porto Alegre Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Study procedures were only begun after written informed consent had been obtained from all participants or their legal guardians.

Participant Recruitment

Patients were eligible for inclusion in the study if they had a diagnosis of hepatic GSD, confirmed by measurement of enzyme activity and/or DNA analysis by massive parallel sequencing using a gene panel designed to all types of GSDs [21], and if they were receiving follow-up at the outpatient metabolic disorders clinic of the Medical Genetics Service, Hospital de Clínicas de Porto Alegre (ATDM-SGM/HCPA, Brazil).

At the time of the study, 42 patients with GSD were seen at ATDM-SGM/HCPA. Of these, 32 (GSD Ia= 18; Ib= 7; III= 3; IXa= 3; IXb= 1; females= 17), from 30 unrelated families, were included. Two patients were aged <3 years and were not included due to the technical difficulty of blood sampling.

One patient refused to participate, and seven did not attend their visits during the recruitment period. The ATDM-SGM/HCPA follow-up protocol includes 3- to 6-monthly visits. Tests considered necessary for assessment of metabolic control (glucose, triglyceride levels, total cholesterol, lactate) are ordered at each visit, while other tests, including abdominal imaging, are performed once yearly.

For comparison of IL-6 levels, eight parents of patients, with a median age of 33.5 years (IQR=27; 37.5), were also included in analyses (GSD Ia= 3; Ib= 3; III= 1; IXb= 1; females= 6).

The exclusion criteria were that neither patients nor parents could present with comorbidities known to affect cytokine levels (e.g., autoimmune diseases). Known complications of GSD that are associated with elevated cytokine levels, such as inflammatory bowel disease, were not among the criteria for exclusion.

Sample Collection

In the morning hours, blood samples from patients (for hepcidin, IL-6, iron, ferritin, and transferrin measurement) and parents (for IL-6 measurement) were collected into heparin sodium tubes. Blood samples were chilled immediately after collection and plasma separated by centrifugation (3000 rpm, 20 min, 4°C) within 30 minutes of collection. Plasma samples were than aliquoted and immediately frozen at -80 °C until the time of testing.

Laboratory Evaluation

Plasma hepcidin levels were measured using a commercially available enzyme-linked immunoassay kit (Hepcidin-25 [human] ELISA Kit, Peninsula Laboratories International, Inc., USA). Plasma IL-6 levels were also measured with a commercially available ELISA kit (IL-6 [Human] ELISA Kit, Invitrogen Corporation, USA). Both hepcidin and IL-6 tests were performed in duplicate and the average of the two measurements taken into account for analysis. No subject had a ≥30% difference in levels between test duplicates.

Iron, ferritin, and transferrin measurements were performed by the HCPA clinical laboratory, and transferrin saturation values were derived using the formula (iron/[transferrin × 1.28]) × 100 [22].

Anthropometric Evaluation

Weight and height were measured on the day of blood draws and used to calculate the BMI, using the formula BMI = (weight [kg]/height [m]^2). Classification of nutritional status was based on BMI z scores calculated in the *Anthro plus* v.1.0.4 software environment. Individuals were classified as underweight, normal weight, overweight, or obese, as age-appropriate, using World Health Organization (WHO) criteria [23].

Chart Review

Data on other clinical, biochemical, and treatment-related variables, including presence or absence of inflammatory bowel disease defined by clinical evaluation, were collected through a chart review. Complete blood count, AST, ALT, vitamin B12, lactate, glucose, triglyceride, and total cholesterol measurements were considered available if performed within 3 months before or after study inclusion; in all instances, the most recent value was used for analyses. Imaging (abdominal ultrasound or magnetic resonance imaging) was considered acceptable if performed no more than 1 year before or after. The median time elapsed in days from sample collection for AST/ALT measurement to sample collection for iron and hepcidin/IL-6 measurement was 2.5 days; 7 days for vitamin B12; 0.5 days to 1 day for cholesterol, triglycerides, glucose, and lactate; and 7.5 days for complete blood count. The median time elapsed in months between most recent imaging and sample collection for the present study was 4.4 months.

The diagnosis of anemia in patients was also based on WHO recommendations, using the following reference ranges of Hb levels (g/dL): a) children aged 6–59 months: >11.0 = normal; 10.0–10.9 = mild anemia; 7.0–9.9 = moderate anemia; <7 = severe anemia; b) children aged 5–11 years: >11.5 = normal; 11.0–11.4 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia; c) children aged 12–14 years: >12.0 = normal; 11.0–11.9 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia; d) females: >12.0 = normal; 11.0–11.9 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia; e) males: >13.0 = normal; 11.0–12.9 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia.

The following reference values were used for the other markers of interest: Mean corpuscular volume (MCV): a) children aged 2–12 years:82–95 fL; b) females:82–96 fL; b) males:83–98 fL. Ferritin: a) females:9–120 ng/mL; b) males:18–370 ng/mL; Iron: a) females:49–151 µg/dL; b) males:53–167 µg/dL. Transferrin saturation.20–50%. Transferrin:200–360 mg/dL. Vitamin B12:180–900 pg/mL.

Statistical Analysis

The descriptive analysis included absolute and relative frequencies. Due to the small sample size, continuous variables were expressed as median and interquartile range (IQR).

Due to the clinical and biochemical similarities, patients with GSD III and IX were grouped for statistical analysis. For comparison between three groups, continuous variable were analyzed using the Kruskal-Wallis test followed by Bonferroni-Dunn post-hoc analysis; while for the comparison between two groups the Mann-Whitney *U* test was used. Categorical variable were compared using Fisher's exact test ($p \leq 0.05$). Correlations were analyzed using Spearman coefficients. The significance level was set at 5%. Statistical analysis was carried out in the *Statistical Package for the Social Sciences* (SPSS) version 22.0 software environment (SPSS Inc., Chicago, IL).

Results

The patients' clinical and laboratory characteristics are summarized in Table 2. Parental consanguinity was present in 7 of 30 families (23.3%).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index.

Reference ranges: hematocrit: a) females: 37-47% b) males: 42-50%; hemoglobin: a) females: 12-16% b) males: 14-18%; MCV: a) children aged 2–12 years:82–95 fL; b) females:82–96 fL; b) males:83–98 fL; platelets: 150-450 μL; glucose: 70-99 mg/dL; triglycerides: <150 mg/dL; total cholesterol: <200 mg/dL; lactate:0,7-1,8 mmol/L; ASL: 10-40 U/L; ALT: 10-40 U/L; Ferritin: a) females:9–120 ng/mL; b) males:18–370 ng/mL. Iron: a) females:49–151 μg/dL; b) males:53–167 μg/dL; Transferrin saturation.20–50%. Transferrin:200–360 mg/dL. Vitamin B12:180–900 pg/mL.

At the time of enrollment, all patients were on uncooked cornstarch therapy. Regarding medications, five patients were on ferrous sulfate (Table 3); five, all with GSD Ib, were in use of granulocyte colony-stimulating factor; 19 of 32 (GSD Ia= 11; Ib= 5; III= 2; IXa=1) were taking a multivitamin; and four, all with GSD Ib, were taking anti-inflammatory (aminosalicylates).

Nine patients (30%) were anemic (Table 3). Four had mild anemia and five had moderate anemia. Five of 32 (15.6%) had inflammatory bowel disease (all with GSD Ib); five of 28 (17.8%) had adenomas (all in the GSD Ia group). Finally, 22 of 32 (68.8%) were classified as having excess weight (GSD Ia= 14; GSD Ib= 5; GSD III= 2; GSD IX= 1): nine as overweight and 13 as obese. No patient had vitamin B12 deficiency.

Analysis of iron metabolism parameters

Eight patients (25%) had hyperferritinemia; of these, only one had elevated transferrin saturation levels, for which hereditary hemochromatosis was excluded. Five of these patients were anemic (Table 3).

Regarding IL-6, the median value measured in patients was 2.33 pg/mL (IQR=1.55; 3.65), versus 1.26 pg/mL (IQR=1.03; 1.85) in patients' parents (p=0.003). Statistical significance remained even after removing from analysis all five patients who were on iron supplementation (data not shown).

Figure 1 illustrates hepcidin and IL-6 levels, stratified by GSD type. There were no statistically significant between-group differences in hepcidin levels (p=0.055). IL-6 levels differed across GSD types (p=0.022). Patients with GSD Ib had higher values than did those with GSD Ia and those with GSD III/IX.

Significant correlations are shown in Figure 2. Hepcidin correlated with lactate levels only (r=0.43; p=0.024). IL-6 correlate with hematocrit (r=-041; p=0.027), iron (r=-0.58; p=0.001), transferrin (r=-0.57; p=0.001) and Hb (r=-0.57; p=0.001). Hb correlate with iron (r=0.55; p=0.001). There was no correlation between hepcidin and IL-6 levels (r=0.319; p=0.081).

There is a statistically significant difference between the three groups for iron concentration (p=0.004; p<0.01 for GSD Ib *vs* GSD III/IX), transferrin saturation (p=0.009; p<0.01 for GSD Ib *vs* GSD III/IX), and IL-6 (p=0.023; p<0.05 for GSD Ia *vs* Ib). There was no statistical difference between GSD groups regarding hemoglobin (p=0.083), ferritin (p=0.387), and transferrin (p=0.350).

Table 2. Summary of patient characteristics.

Variable	Value (median, IQR)	n (32)
Age (years)	9.5 (8.0; 16.75)	32
Weigth (kg)	40.8 (25.7; 62.57)	32
Height (cm)	138 (118; 154)	32
BMI (kg/m2)	22.6 (18.3; 27.0)	32
Hematocrit (%)	36.1 (34.0; 38.4) / 37.5 (35.6; 38.5)*	30/25*
Hemoglobin (g/dL)	12.3 (11.3; 13.4) / 12.6 (11.8; 13.6)*	30/25*
MCV (fL)	81.0 (77.9; 84.3) / 80.4 (77.8; 84.5)*	30/25*
Platelets (μL)	392.5 (318.8; 462.0)	28
Glucose (mg/dL)	87.0 (80.5; 95.5)	29
Triglycerides (mg/dL)	212.5 (93.8; 386.5)	26
Total Cholesterol (mg/dL)	174.5 (150.8; 200.0)	26
Lactate (mmol/L)	2.0 (1.28; 2.73)	26
AST (U/L)	27.0 (23.0; 57.0)	26
ALT (U/L)	24.0 (16.0 63.5)	26
Iron (μg/dL)	67.0 (44.3; 89.8) / 68.0 (52.0; 92.0)*	32/27*
Ferritin (mg/mL)	90.8 (55.6; 164.2) / 85.6 (55.1; 122.2)*	32/27*
Transferrin (mg/dL)	313.5 (272.5; 334.8) / 318.0 (282.0; 335)*	32/27*
Transferrin saturation (%)	15.6 (12.2; 23.2) / 17.5 (12.9; 25.3)*	32/27*
Vitamin B12 (pg/mL)	436.5 (335.5; 627.5) / 419.0 (330.0; 572.0)*	20/15*

* Excluding patients on oral iron supplementation.

Table 3. Characteristics of GSD patients with anemia (n=9/30).

Patient	Sex	Age (years)	Type of GSD	IBD	Liver adenoma	Overweight*	Multivitamin supplementation	Oral iron supplementation	Hb (g/dL)	MCV (fL)	Iron (µg/dL)	Ferritin (ng/mL)	Transferrin (mg/dL)	Transferrin sat. (%)	Vitamin B12 (pg/mL)	IL-6 (pg/mL)	Hepcidin (ng/mL)
1	F	17	Ia	No	Yes	No	No	Yes	9.1	92.8	93	199.7	598	12.1	441	1.7	70.27
2	F	8	Ia	No	No	Yes	No	Yes	10.5	75	21	27.2	290	5.7	269	7.7	65.2
3	M	10	Ia	No	No	Yes	No	No	11.3	77.9	66	241.8	355	14.5	432	1.82	30.51
4	F	32	Ib	Yes	NA	Yes	Yes#	Yes (prophylactic)	7.8	82.6	29	166.7	182	12.4	650	15.43	77.24
5	M	3	Ib	No	No	Yes	No	No	10.8	75.3	17	44.1	238	5.6	572	16.97	53.97
6**	M	14	Ib	Yes	No	Yes	Yes¶	No	11.3	80	34	64.4	402	6.6	NA	3.52	6.69
7**	F	3	Ib	Yes	No	No	No	No	9.2	71	14	164.8	291	3.8	NA	10.97	92.67
8	F	13	Ib	Yes	No	Yes	Yes#	Yes (prophylactic)	11.6	81	79	216	277	22.3	641	3.91	86.05
9	F	6	Ib	No	No	No	Yes¶	Yes	9.7	82.6	28	452.5	261	8.4	932	10.01	69.48

F, female; M, male; NA, not available;
GSD, glycogen storage disease; IBD, inflammatory bowel disease; IL-6: interleukin-6; Transferrin sat., transferrin saturation.
** Subjects were classified as overweight on the basis of body mass index, in accordance with World Health Organization recommendations.*
***Iron had been prescribed, but the patient did not take it.*
#Vitamins A, B1, B2, B3, B5, B6, B9, B12, C, D, E, H, and K, calcium, chloride, copper, chromium, iron, phosphorus, iodine, magnesium, manganese, molybdenum, potassium, selenium, zinc.
¶Vitamins A, B1, B2, B6, B12, C, D3, E, nicotinamide, folic acid, panthenol.
Reference ranges:
Hb: a) children aged 6–59 months: >11.0 = normal; 10.0–10.9 = mild anemia; 7.0–9.9 = moderate anemia; <7 = severe anemia; b) children aged 5–11 years: >11.5 = normal; 11.0–11.4 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia; c) children aged 12–14 years: >12.0 = normal; 11.0–11.9 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia; d) females: >12.0 = normal; 11.0–11.9 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia; e) males: >13.0 = normal; 11.0–12.9 = mild anemia; 8.0–10.9 = moderate anemia; <8 = severe anemia.
MCV: a) children aged 2–12 years:82–95 fL; b) females:82–96 fL; b) males:83–98 fL.
Ferritin: a) females:9–120 ng/mL; b) males:18–370 ng/mL.
Iron: a) females:49–151 µg/dL; b) males:53–167 µg/dL.
Transferrin saturation.20–50%.
Transferrin:200–360 mg/dL.
Vitamin B12:180–900 pg/mL
Hepcidin: 0.02-25 pg/ml.
IL-6: 5-15 pg/m

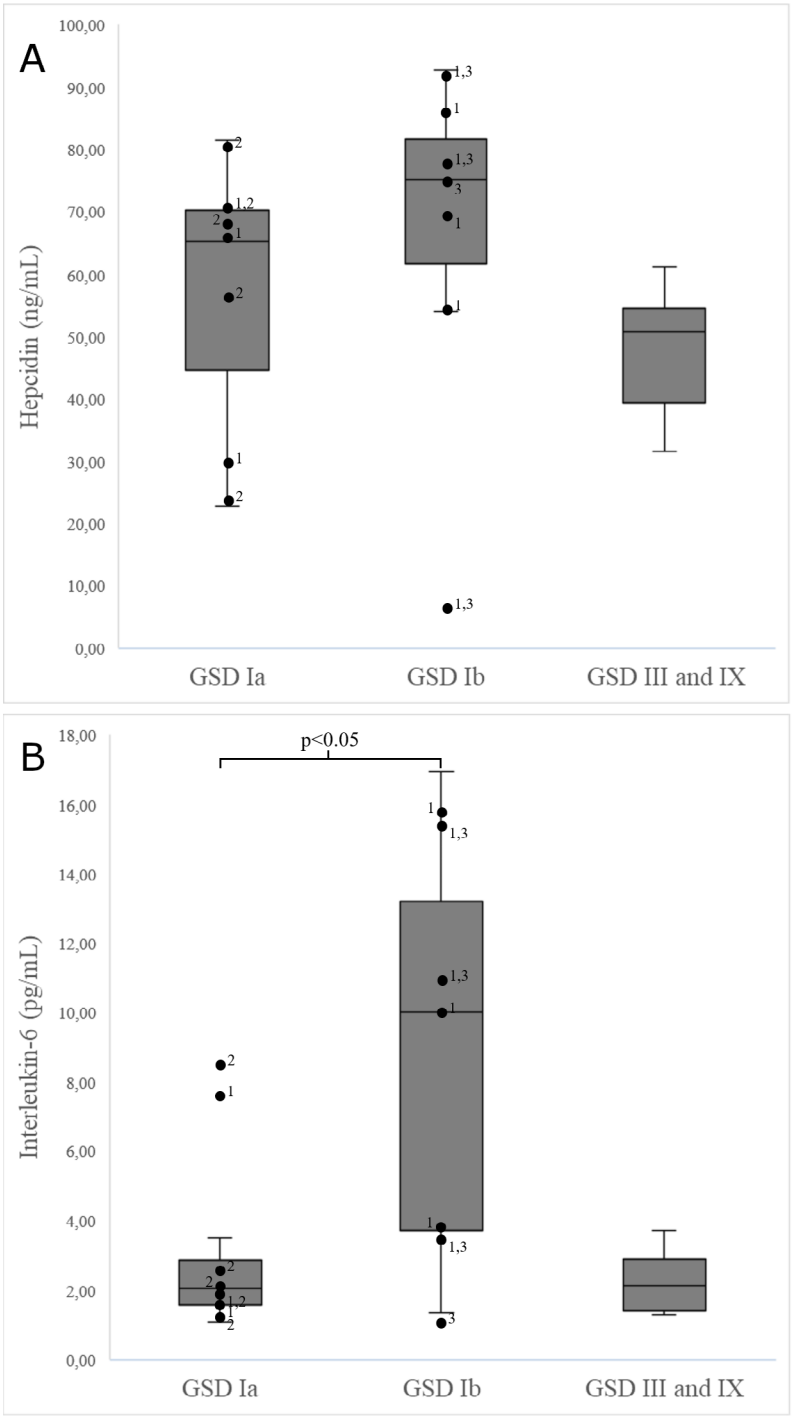

Figure 1. A. Comparison of hepcidin levels stratified by type of GSD; B. Comparison of interleukin-6 levels stratified by type of GSD. GSD Ia, n=18; Ib, n=7; III IX, n=7.

● *patient with (1) anemia, (2) IBD, and (3) adenoma; GSD, glycogen storage disease. Reference range: Hepcidin: 0.02-25 pg/ml and IL-6: 5-15 pg/ml.*

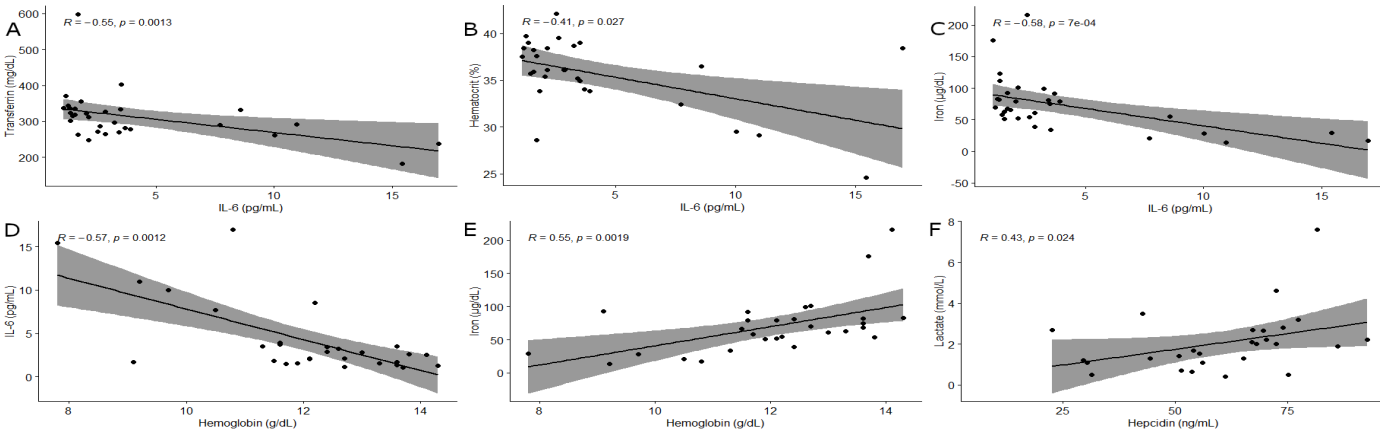

Figure 2. Statisticaly significant correlations between IL-6, Hepcidin and Hemoglobin with the others biomarkers analyzed. Spearman correlation between IL-6 and transferrin (A), hematocrit (B), iron (C), and hemoglobin (D); between hemoglobin and iron (E); and between hepcidin and lactate (F).

Correlation with Clinical Variables

Overweight and obese patients did not differ regarding the variables of interest, nor did patients with adenoma and those without (data not shown). Analysis of patients with inflammatory bowel disease revealed a significant difference only in hepcidin values, which were higher in patients with inflammatory bowel disease than in those without this condition (p=0.003), but not with IL-6 (p=0.249). Patients with anemia had higher IL-6 values than non-anemic ones (p=0.002).

Discussion

To the best of our knowledge, this was the first study to investigate the association between plasma levels of hepcidin and IL-6 in a relatively large cohort of patients with hepatic GSD. Our findings confirmed that anemia is especially prevalent in GSD Ib, the type of GSD most commonly associated with inflammatory bowel disease. Furthermore, the IL-6 concentrations found strengthen the hypothesis that the presence of anemia in patients with the "inflammatory" GSD Ib is influenced by the underlying inflammatory state, since these patients showed higher levels of IL-6 in comparison to the other types. Probably due to the small size and heterogeneity of our sample, we did not find some expected associations/correlations such as between hepcidin and IL-6 levels; inflammatory bowel disease and IL-6 levels; and liver adenoma and hepcidin or IL-6 levels.

In the present study, patients with GSD Ib had significantly higher levels of IL-6 than did patients with GSD Ia and GSD III/IX. However, some patients with GSD Ia had IL-6 values like those of patients with GSD Ib. Elevated IL-6 levels are to be expected in chronic inflammation, and may be associated with the fact that inflammatory bowel disease is a frequent finding in GSD Ib. Furthermore, the presence of gastrointestinal symptoms and manifestations of inflammatory bowel disease has been described as a possible finding in GSD Ia [24], and markers of this condition may be present even in asymptomatic patients [25]. In addition, the IL-6 levels measured in patients with hepatic GSD were higher than those of their parents, suggesting a possible increase in this marker in this population. Analyzes involving CRP or sedimentation rates could be informative in this context but were not evaluated in the present sample. A prior study evaluated IL-6 levels by ELISA assay in 27 patients with GSD Ia (mean age=15 years, range= 2–35), fourteen patients with GSD III/VI (mean age=16 years, range= 4–28), and 30 healthy adult controls (mean age=28 years, range=22–38).

No positive correlation between hepcidin and IL-6 was found in the present study, although the p-value found was borderline. Previous studies are ambiguous regarding this finding; some found a positive correlation between hepcidin and IL-6 [17,26,27], whereas others found no significance for this correlation [28-31]. It is important to note that other cytokines may be involved in hepcidin regulation and influence correlation between these variables [18,32]. Another study analyzed the levels of hepdicin and IL-6 in patients with children with chronic liver disease, including six patients with GSD, and no correlation was found between this two variables, although the prevalence of anemia was more frequent in patients with chronic liver disease ($p<0.05$) [33].

Anemia is a common manifestation of inflammatory bowel disease, and its etiology is most often associated with iron deficiency and chronic inflammation. Less common causes of anemia in inflammatory bowel disease are vitamin B12 deficiency and folic acid deficiency [34]. In the present study, most of patients with GSD Ib had anemia associated with inflammatory bowel disease, whereas a smaller portion of patients with GSD Ia were deemed to have anemia. Patients with the other GSD types did not exhibit this condition. A study by Wang et al. [13], with a larger sample (n=195) of U.S. patients with GSD Ia and Ib, found rates of anemia different from those observed in our study, with a higher rate in GSD Ia (41.7% vs. 17.6%) and a lower rate in GSD Ib (71.8% vs 85.7%). According to the same study,

iron deficiency anemia would be more common in preadolescent patients with GSD I, whereas anemia of chronic disease would be more common in adult patients. Due to the small size and relatively young age of our sample, we were unable to test for associations between type of anemia and age. However, it is clear that macrocytic anemia is not a common finding in patients with hepatic GSD; the differential diagnosis is restricted to the causes of microcytic and normocytic anemia in general, and to iron deficiency anemia and anemia of chronic disease in particular. Unfortunately, the ferritin levels may not reflect the iron status of these patients.

Hepcidin, a 25-amino acid peptide hormone encoded by the *HAMP* gene (hepcidin antimicrobial peptide - MIM606464), is involved in the pathogenesis of anemia of chronic disease [20,31,35-38]. This peptide operates through the hepcidin–ferroportin complex, which regulates intracellular and extracellular iron concentrations. Ferroportin is a transmembrane receptor that exports cellular iron. When levels of hepcidin increase, it binds to ferroportin and induces its internalization and degradation. Consequently, iron delivery into plasma is decreased through inhibition of iron absorption by enterocytes in the bowel and inhibition of mobilization of body iron stores [18-20,31,39-41]. Inflammation may induce hepcidin synthesis mediated by cytokines, particularly IL-6, thus leading to the anemia of chronic disease [42-47]. The possibility of involvement of hepcidin in the pathogenesis of anemia in hepatic GSD was raised by Weinstein et al. [16] after finding that five patients with GSD Ia had large hepatic adenomas and severe anemia nonresponsive to iron supplementation. Examination of adenoma tissue from two of these patients revealed increased hepcidin mRNA levels and anemia was found to resolve after adenoma resection or liver transplantation [16]. In the present study hepatic adenomas were observed in five patients with GSD Ia and only one was anemic. Adenomas have been associated with a high rate of microscopic or macroscopic hemorrhage according to the subtype. Large adenomas increase the risk of bleed with or without tumor rupture, may lead to anemia. However, few studies have reported iron deficiency anemia in large adenomas without hemorrhage [48-50].

Hepcidin correlated with lactate levels ($r=0.43$; $p=0.024$). Recently Liu et al. [51] showed that lactate promotes hepatic hepcidin expression through cyclic adenosine monophosphate-PKA-Smad signaling, directly binds to soluble adenylyl cyclase to enhance its enzymatic activity and modulates iron homeostasis by inducing hepatic hepcidin expression in mice. More human studies need to be conducted, but this could be a possible mechanism that explains the correlation between lactate and hepcidin [51].

Conclusions

Our findings suggest that inflammation is related to the occurrence of anemia in GSD Ib. A better understanding of the mechanisms involved in anemia in hepatic GSD may help the future introduction of new therapies and in preventing anemia in these diseases. Meanwhile, we suggest patients with hepatic GSD are regularly monitored in relation to this complication.

Acknowledgments

The authors thank Filippo Vairo, Bruna Bento dos Santos, Kamila Castro, Lilia Refosco, Nataniel Ludwig, and Vitor Mendes. Financial support was provided by the CAPES Foundation/ Ministry of Education of Brazil and CNPq/Ministry of Science, Technology, and Innovation of Brazil [T.N.], Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) [F.S.L., postdoctoral fellowship], CNPq (Chamada Nº 31/2013 – Doenças Metabólicas e Endócrinas), FAPERGS (PPSUS/2013), and FIPE - HCPA (Financiamento e Incentivo à Pesquisa - Hospital de Clínicas de Porto Alegre).

Ethics Approval and Consent to Participate

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Authors' Contributions

TN and IVD - Study conception and design. TN, FSL and MS Conducted experiments. TN, FSL, MS, GMR, DAW, TGJD, CFMS and IVDS Conducted data analysis. TN, FSL, MS, GMR, DAW, TGJD, CFMS and IVDS Participated in writing the manuscript. All authors participated fully in critically revising the manuscript and approved the final version of the manuscript to be published.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Wolfsdorf JI, Weinstein DA. Glycogen storage diseases. *Rev Endocr Metab Disord*. 2003;4(1):95-102. doi:10.1023/a:1021831621210.
2. Beauchamp NJ, Dalton A, Ramaswami U, et al. Glycogen storage disease type IX: High variability in clinical phenotype. *Mol Genet Metab*. 2007;92(1-2):88-99. doi:10.1016/j.ymgme.2007.06.007.
3. Kishnani PS, Austin SL, Arn P, et al. Glycogen storage disease type III diagnosis and management guidelines. *Genet Med*. 2010;12(7):446-463. doi:10.1097/GIM.0b013e3181e655b6.

4. Hicks J, Wartchow E, Mierau G. Glycogen storage diseases: A brief review and update on clinical features, genetic abnormalities, pathologic features, and treatment. *Ultrastruct Pathol*. 2011;35(5):183-196. doi:10.3109/01913123.2011.601404.

5. Dagli A, Sentner CP, Weinstein DA. Glycogen Storage Disease Type III. In: Adam MP, Feldman J, Mirzaa GM, et al, eds. *Glycogen Storage Disease Type III. GeneReviews*. Seattle, WA: University of Washington; 2012:0-00.

6. Bali DS, Chen Y, Goldstein JL. Glycogen Storage Disease Type I. In: Adam MP, Feldman J, Mirzaa GM, et al, eds. *Glycogen Storage Disease Type I. GeneReviews*. Seattle, WA: University of Washington; 2013:0-00.

7. Kishnani PS, Austin SL, Abdenur JE, et al. Diagnosis and management of glycogen storage disease type I: A practice guideline of the American College of Medical Genetics and Genomics. *Genet Med*. 2014;16(11):e1. doi:10.1038/gim.2014.128.

8. Sentner CP, Hoogeveen IJ, Weinstein DA, et al. Glycogen storage disease type III: diagnosis, genotype, management, clinical course and outcome. *J Inherit Metab Dis*. 2016;39(5):697-704. doi:10.1007/s10545-016-9932-2.

9. Chen YT. Glycogen storage diseases. In: Scriver C, Beaudet A, SlyW, et al, eds. *The metabolic and molecular bases of inherited disease*. New York, NY: McGraw-Hill; 2001:1521-1555.

10. Ross KM, Ferrecchia IA, Dahlberg KR, Dambska M, Ryan PT, Weinstein DA. Dietary Management of the Glycogen Storage Diseases: Evolution of Treatment and Ongoing Controversies. *Adv Nutr*. 2020;11(2):439-446. doi:10.1093/advances/nmz092.

11. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP. Glycogen storage disease type I: Diagnosis, management, clinical course and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). *Eur J Pediatr*. 2002;161 Suppl 1:S20-S34. doi:10.1007/s00431-002-0999-4.

12. Talente GM, Coleman RA, Alter C, et al. Glycogen storage disease in adults. *Ann Intern Med*. 1994;120(3):218-226. doi:10.7326/0003-4819-120-3-199402010-00008.

13. Wang DQ, Carreras CT, Fiske LM, et al. Characterization and pathogenesis of anemia in glycogen storage disease type Ia and Ib. *Genet Med*. 2012;14(9):795-799. doi:10.1038/gim.2012.41.

14. Sato H, Takase K, Kin S. Successful treatment of refractory anemia in a patient with glycogen storage disease type ia undergoing hemodialysis. *Cureus*. 2022;14(6):e26213. doi:10.7759/cureus.26213.

15. Colonetti K, Pinto E Vairo F, Siebert M, et al. Cytokine profiling in patients with hepatic glycogen storage disease: Are there clues for unsolved aspects? *Cytokine*. 2023;162:156088. doi:10.1016/j.cyto.2022.156088.

16. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, Andrews NC. Inappropriate expression of hepcidin is associated with iron refractory anemia: Implications for the anemia of chronic disease. *Blood*. 2002;100(10):3776-3781. doi:10.1182/blood-2002-04-1260.

17. Semrin G, Fishman DS, Bousvaros A, et al. Impaired intestinal iron absorption in Crohn's disease correlates with disease activity and markers of inflammation. *Inflamm Bowel Dis*. 2006;12(12):1101-1106. doi:10.1097/01.mib.0000235097.86360.04.

18. Ganz T. Systemic iron homeostasis. *Physiol Rev*. 2013;93(4):1721-1741. doi:10.1152/physrev.00008.2013.

19. Nemeth E, Ganz T. Hepcidin-Ferroportin Interaction Controls Systemic Iron Homeostasis. *Int J Mol Sci*. 2021;22(12):6493. doi:10.3390/ijms22126493.

20. Zhao N, Zhang AS, Enns CA. Iron regulation by hepcidin. *J Clin Invest*. 2013;123(6):2337-2343. doi:10.1172/JCI67225.

21. Sperb-Ludwig F, Pinheiro FC, Bettio Soares M, et al. Glycogen storage diseases: Twenty-seven new variants in a cohort of 125 patients. *Mol Genet Genomic Med*. 2019;7(11):e877. doi:10.1002/mgg3.877.

22. Punnonen K, Irjala K, Rajamaki A Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood*. 1997;89:1052-1057.

23. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Genebra, CH: World Health Organization; 2011.

24. Lawrence NT, Chengsupanimit T, Brown LM, Derks TG, Smit GP, Weinstein DA. Inflammatory Bowel Disease in Glycogen Storage Disease Type Ia. *J Pediatr Gastroenterol Nutr*. 2017;64(2):e52-e54. doi:10.1097/MPG.0000000000000592.

25. Lawrence NT, Chengsupanimit T, Brown LM, Weinstein DA. High incidence of serologic markers of inflammatory bowel disease in asymptomatic patients with glycogen storage disease type Ia. *JIMD Rep*. 2015;24:123-128. doi:10.1007/8904_2015_452.

26. Hohaus S, Massini G, Giachelia M, et al. Anemia in Hodgkin's lymphoma: The role of interleukin-6 and hepcidin. *J Clin Oncol*. 2010;28(15):2538-2543. doi:10.1200/JCO.2009.27.6873.

27. Li H, Feng SJ, Su LL, Wang W, Zhang XD, Wang SX. Serum hepcidin predicts uremic accelerated atherosclerosis in chronic hemodialysis patients with diabetic nephropathy. *Chin Med J (Engl)*. 2015;128(10):1351-1357. doi:10.4103/0366-6999.156781.

28. Matsumoto M, Tsujino T, Lee-Kawabata M, et al. Iron regulatory hormone hepcidin decreases in chronic heart

failure patients with anemia. *Circ J*. 2010;74(2):301-306. doi:10.1253/circj.cj-09-0663.

29. Eleftheriadis T, Pissas G, Remoundou M, et al. Ferroportin in monocytes of hemodialysis patients and its associations with hepcidin, inflammation, markers of iron status and resistance to erythropoietin. *Int Urol Nephrol*. 2014;46(1):161-167. doi:10.1007/s11255-013-0497-9.

30. Łukaszyk E, Łukaszyk M, Koc-Żórawska E, Tobolczyk J, Bodzenta-Łukaszyk A, Małyszko J. Iron status and inflammation in early stages of chronic kidney disease. *Kidney Blood Press Res*. 2015;40(4):366-373. doi:10.1159/000368512.

31. Yacoub MF, Ferwiz HF, Said F. Effect of interleukin and hepcidin in anemia of chronic diseases. *Anemia*. 2020;2020:3041738. doi:10.1155/2020/3041738.

32. Kanamori Y, Murakami M, Sugiyama M, Hashimoto O, Matsui T, Funaba M. Hepcidin and IL-1β. *Vitam Horm*. 2019;110:143-156. doi:10.1016/bs.vh.2019.01.007.

33. Cakir M, Erduran E, Turkmen ES, et al. Hepcidin levels in children with chronic liver disease. *Saudi J Gastroenterol*. 2015;21(5):300-305. doi:10.4103/1319-3767.166205.

34. Kaitha S, Bashir M, Ali T. Iron deficiency anemia in inflammatory bowel disease. *World J Gastrointest Pathophysiol*. 2015;6(3):62-72. doi:10.4291/wjgp.v6.i3.62.

35. Roy CN, Weinstein DA, Andrews NC. 2002 E. Mead Johnson Award for Research in Pediatrics Lecture: the molecular biology of the anemia of chronic disease: A hypothesis. *Pediatr Res*. 2003;53(3):507-512. doi:10.1203/01.PDR.0000049513.67410.2D.

36. Jankowska EA, von Haehling S, Anker SD, Macdougall IC, Ponikowski P. Iron deficiency and heart failure: Diagnostic dilemmas and therapeutic perspectives. *Eur Heart J*. 2013;34(11):816-829. doi:10.1093/eurheartj/ehs224.

37. Mei S, Wang H, Fu R, et al. Hepcidin and GDF15 in anemia of multiple myeloma. *Int J Hematol*. 2014;100(3):266-273. doi:10.1007/s12185-014-1626-7.

38. Mahajan G, Sharma S, Chandra J, Nangia A. Hepcidin and iron parameters in children with anemia of chronic disease and iron deficiency anemia. *Blood Res*. 2017;52(3):212-217. doi:10.5045/br.2017.52.3.212.

39. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood*. 2003;102(3):783-788. doi:10.1182/blood-2003-03-0672.

40. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004;113(9):1271-1276. doi:10.1172/JCI20945.

41. Kali A, Charles MV, Seetharam RS. Hepcidin - A novel biomarker with changing trends. *Pharmacogn Rev*. 2015;9(17):35-40. doi:10.4103/0973-7847.156333.

42. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;306(5704):2090-2093. doi:10.1126/science.1104742.

43. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. *Blood*. 2006;108(9):3204-3209. doi:10.1182/blood-2006-06-027631.

44. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. *Nat Rev Immunol*. 2015;15(8):500-510. doi:10.1038/nri3863.

45. Anderson GJ, Frazer DM. Current understanding of iron homeostasis. *Am J Clin Nutr*. 2017;106(Suppl 6):1559S-1566S. doi:10.3945/ajcn.117.155804.

46. Ganz T. Iron and infection. *Int J Hematol*. 2018;107(1):7-15. doi:10.1007/s12185-017-2366-2.

47. Nemeth E, Ganz T. Hepcidin and Iron in Health and Disease. *Annu Rev Med*. 2023;74:261-277. doi:10.1146/annurev-med-043021-032816.

48. Koh YK, Yoon SH, Kang SH, et al. Large hepatocellular adenoma presenting with iron deficiency anemia: A case report. *Clin Pediatr Hematol Oncol*. 2023;30(1):25-29. doi:10.15264/cpho.2023.30.1.25.

49. McDermott C, Ertreo M, Jha R, et al. Risk factors for bleeding hepatocellular adenoma in a United States cohort. *Liver Int*. 2022;42(1):224-232. doi:10.1111/liv.15087.

50. Beaufrère A, Paradis V. Hepatocellular adenomas: Review of pathological and molecular features. *Hum Pathol*. 2021;112:128-137. doi:10.1016/j.humpath.2020.11.016.

51. Liu W, Zhang S, Li Q, et al. Lactate modulates iron metabolism by binding soluble adenylyl cyclase. *Cell Metab*. 2023;35(9):1597-1612.e6. doi:10.1016/j.cmet.2023.06.017.