Ironing out pathophysiological aspects of Gaucher disease

Regenboog, M.

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Serum hepcidin levels and iron homeostasis in Gaucher disease type 1

M Regenboog, L van Dussen, RPL van Swelm, DW Swinkels, CEM Hollak

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Abstract

Gaucher disease (GD) is associated with altered iron homeostasis, as reflected by hyperferritinemia and iron storage in Gaucher cells. Persistent storage of iron despite treatment may be related to increased cancer risk. To understand the cause of hyperferritinemia, we studied hepcidin, the key regulator of iron homeostasis, in relation to iron status and well established disease markers. We analyzed haemoglobin, total iron, transferrin, total iron binding capacity, transferrin saturation and hepcidin levels in 40 (38 treated) type 1 GD patients (GD1) and compared them to 40 healthy controls. Liver iron concentrations were determined using magnetic resonance imaging (MRI). The findings are studied in relation to chitotriosidase, reflecting the overall disease burden in GD. No significant difference was found in absolute hepcidin levels between GD1 patients and healthy controls. Ferritin levels were significantly elevated in GD1 patients (median level 333 µg/l (range 40-1520)) versus 152 µg/l (range 10-612) in controls, $p<0.0001$). Despite high ferritin levels, hepcidin levels were in the normal range, but correlated with ferritin levels and liver R2* values on MRI. GD1 patients with persistent hyperferritinemia despite therapy exhibit higher hepcidin levels and increased amounts of iron storage in the liver on MRI as compared to patients with normal ferritin levels. Hepcidin- and ferritin levels did not correlate with chitotriosidase. Hepcidin levels in GD1 patients are low but correlate with ferritin and the presence of iron storage in the liver, suggesting an iron distribution disorder. Transferrin saturations are normal in the presence of hyperferritinemia. Although the hepcidin response is insufficient, some feedback regulation is intact as hyperferritinemic patients show higher hepcidin levels, with no correlation to overall residual disease. We hypothesize that these patients, with persisting high ferritins, may be at the highest risk for late complications such as cancer.

Introduction

Gaucher disease (GD; OMIM #230800) is a lysosomal storage disorder in which deficient activity of the enzyme glucocerebrosidase (GBA; EC 3.2.1.45) leads to accumulation of glucocerebroside in lysosomes of tissue macrophages. Lipid-laden pathological storage macrophages are a hallmark of the disorder and predominantly reside in liver, spleen and bone marrow [1,2]. GD is phenotypically classified in three subtypes. Type 1 (GD1) is the most prevalent form, and is characterized by visceral and/or skeletal involvement without the classical neurological involvement as seen in type 2 and 3 GD [3,4]. Over 200 different mutations in the gene encoding GBA have been identified [5].

For more than two decades, GD has been treatable with enzyme replacement therapy (ERT) [6] which has proven to be very effective. It ameliorates clinical signs and symptoms and has a positive effect on quality of life of GD patients [7-9]. More recently substrate inhibitors, acting through inhibition of glucosylceramide synthase (GCS), as well as alternative enzyme replacement therapies have become available [10-12]. The window of opportunity for treatment is very wide, since in many patients the disease sequelae are reversible with therapy. However, some late complications may occur despite treatment, including persisting bone disease, fibrosis of the liver or susceptibility to cancers [13-15]. It is likely that advanced disease, including prior splenectomy, impacts on these risks. The GBA1 genotype plays a limited role in predicting the disease course [2,16]. Presumably, individual susceptibility to complications is determined by multiple factors, including potential modifier genes e.g. implicated in substrate production, inflammation or iron homeostasis [17].

Evidence for an altered iron homeostasis goes back to early observations of iron particles in the pathological storage cells [18-20]. This, however, is not a specific hallmark of GD: sequestration of iron in macrophages is a feature of several inflammatory conditions, reflected by elevated serum ferritin levels [21-23]. At initial presentation of a GD patient, hyperferritinemia is a common finding. Treatment of GD decreases ferritin levels, although some patients may still exhibit high levels despite prolonged treatment [24-28]. These persisting high ferritin levels probably indicate residual Gaucher cells with iron overload [29], which does not necessarily correlate with residual disease as evidenced by elevated chitotriosidase levels [30].

We recently reviewed the literature on iron metabolism in GD and hypothesized that altered iron metabolism in GD may contribute to the occurrence of associated conditions and complications such as cancer, metabolic syndrome and perhaps Parkinson's disease [31]. However, the exact mechanism and consequences of dysregulation of iron homeostasis in GD is still obscure. Over the past years, our understanding in the field of iron metabolism and regulation in general has improved due to unraveling of main components involved [32]. Iron is stored as ferritin in the cytoplasm of hepatocytes and macrophages with hepcidin identified as the key player to maintain systemic iron homeostasis [33,34]. A number of factors influence hepcidin levels [35]. Inflammatory conditions, for instance, result in upregulation of hepcidin secretion with subsequent excess iron storage in macrophages. Gaucher cells are hypothesized to elicit an inflammatory response from surrounding macrophages. One might therefore argue that this chronic inflammatory state could lead to high hepcidin levels in GD patients. Iron loading as a result of transfusions may contribute to high hepcidin levels as well. On the other hand, anemia and increased erythropoiesis in GD may lead to suppression of hepcidin production. In line with this, contrasting findings regarding hepcidin levels in GD patients have been reported [27,36,37].

The aim of this study was to better understand hyperferritinemia by studying the relation of ferritin with hepcidin and other parameters of iron metabolism in the Dutch cohort of GD patients and healthy controls. Iron parameters were also studied in relation to key disease parameters and discussed in the context of earlier and contradictory results on hepcidin measurements in this population.

Methods

Participants

Forty type 1 GD patients and 40 healthy controls, matched for age and sex, were enrolled in this study. This population was also described in a separate study covering imaging data [29]. All GD1 patients were followed for several years in the Academic Medical Center (AMC) Amsterdam, which is the national referral center for GD in the Netherlands. In all patients, the diagnosis of GD1 is made based on deficient glucocerebrosidase enzyme activity and genotyping at time of first presentation. Most patients were treated with enzyme replacement therapy (ERT) or substrate reduction therapy (SRT). The study was approved by the Medical Ethical Committee of the AMC Amsterdam and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical parameters

Clinical parameters recorded for every participant in this study include age, gender, body mass index (BMI), smoking history and alcohol use. GD1 patients were further characterized by splenectomy and treatment status, duration of treatment, genotype, chitotriosidase levels and platelet counts. A presence of a history of blood transfusions was noted for GD1 patients.

Laboratory assessments

Blood samples (non-fasting) were collected between May 2014 and September 2015 on the same day as magnetic resonance examination and directly analyzed in the laboratory of the AMC in Amsterdam. Haemoglobin (Hb) concentration is determined by spectrophotometry (Sysmex XE-5000, Goffin Meyvis BV, the Netherlands). Mean corpuscular volume (MCV) was calculated ((Ht/RBC) x 1000). Ferritin levels were analyzed by using an electrochemiluminescense immunoassay (e602, Roche Diagnostics), total iron concentration by colorimetric measurements (c502, Roche Diagnostics) and transferrin by immunoturbidimetry (c702, Roche Diagnostics). Total iron binding capacity (TIBC) is calculated by multiplying transferrin by 25.2 (TIBC (μmol/L) = transferrin (g/L) x 25.2). Transferrin saturation (Tsat) is calculated as follows: Tsat = (total iron concentration (μmol/L) / TIBC) x 100.

In GD1 patients, ferritin levels before start of therapy were also extracted from medical files to study the course of ferritin levels in response to ERT or SRT. Patients with persisting

high ferritin levels despite therapy (defined as ferritin levels >300 µg/L for males and >250 µg/L for females) were compared to patients with normalized ferritin levels. Differences in iron profile, hepcidin and chitotriosidase levels between these groups were examined. Patients who were on treatment (ERT or SRT) for less than two years were excluded from this subgroup analysis. Chitotriosidase enzyme activity was determined as part of routine follow-up and used as marker of disease [38]. Chitotriosidase levels were multiplied by 2 for patients with heterozygosity for the 24-bp duplication in the CHIT1-gene [39].

Hepcidin analysis

Separate samples for hepcidin analysis were stored in -80 °C and send out for analysis after including all participants. Serum hepcidin measurements were performed in September 2015 (testing laboratory: hepcidinanalysis.com, Nijmegen, the Netherlands) by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (WCX-TOF MS) [40]. An internal standard (synthetic heavy hepcidin-25 stable isotope stable isotope $^{+40}$; custom made Peptide International Inc.) was used for quantification [41]. Peptide spectra were generated on a Microflex L T matrix-enhanced laser desorption/ionisation TOF MS platform (Bruker Daltonics). Plasma hepcidin-25 concentrations were expressed as nmol/L (nM). The lower limit of detection of this method was 0.5 nM. The hepcidin/ferritin ratio and TSAT/hepcidin ratio were reported to be able to interpret the hepcidin values in context of iron regulation.

Liver iron concentration

Liver iron concentrations are measured by magnetic resonance imaging (MRI) using a spoiled gradient recalled echo sequence on a 1.5 Tesla machine (Siemens Avanto, Siemens AG, Erlangen, Germany) as reported earlier [29]. R2* relaxation rates (in Hertz, Hz) of the liver are calculated and reported as surrogate marker of liver iron concentration [42,43].

Statistical analysis

Comparisons of the haematological and iron-metabolism parameters between groups (patients versus controls) were made using paired analysis. The paired t-test was used for normally distributed data and the Wilcoxon signed rank test was applied for variables not passing the normality check. Since the detection limit of hepcidin levels was 0.5 nM, participants with a hepcidin level below this detection limit were recorded as having a hepcidin level of 0.5 nM. Patients with persisting high ferritin levels despite therapy were compared to patients with normalized ferritin levels using the Mann-Whitney U test. Correlations are described using Spearman's rho. A p-value <0.05 was considered

statistically significant. All analyses were performed with IBM SPSS version 23.0 (SPSS Inc. Chicago, Illinois, USA) and GraphPad Prism 7.01 (Graphpad Software Inc., La Jolla, CA, USA).

Results

A summary of demographic characteristics of all participants is given in table 1. Of the 40 GD1 patients, 10 patients were splenectomized in the past. The majority of patients (95%) was treated with ERT (n=35) or SRT (n=3) for multiple years.

In table 2 the results of laboratory analysis and liver iron levels on MRI are provided. Median haemoglobin levels and transferrin concentrations are lower in patients. Seven patients have a mild anaemia according to reference values (three females between 11.0-11.8 g/dL, four males between 13.0-13.4 g/dL). Hepcidin levels did not differ between patients and healthy controls (figure 1A). One patient was found to have an increased hepcidin of 20.3 nM (upper limit of normal for this patient: 15.6 nM). Ferritin levels were significantly higher in GD1 patients as compared to controls (figure 1C). The hepcidin-ferritin ratio was significantly lower in GD1 patients (median 12.9 pmol/µg) as compared to healthy controls (median 33.6 pmol/µg), as is also depicted in figure 1B.

A total number of 22 patients (55%) had elevated ferritin levels (>300 µg/L for males and >250 µg/L for females). Ferritin levels at baseline (before start of ERT/SRT) were elevated in 33 out of 39 patients (85%). Pretreatment ferritin level was missing in one patient. In figure 2 the course of ferritin levels in individual patients pretreatment (for untreated patients: ferritin level at first presentation) as compared to current level is shown. Of the 22 patients with current elevated ferritin levels, i.e. persistent hyperferritinemia despite therapy, 12 patients had ferritin levels more than two times the upper limit of normal (ULN; >600 µg/L for males and >500 µg/L for females).

Hepcidin levels correlated with ferritin levels (Spearman's rho 0.64, $p<0.0001$, figure 3A), and with liver R2* values on MRI (Spearman's rho 0.59, $p<0.0001$, figure 3B). No correlation was found between hepcidin levels and chitotriosidase. Ferritin levels showed a correlation with liver R2* values (Spearman's rho 0.81, $p<0.0001$, figure 3C). Ferritin levels and hepcidin-ferritin ratio's did not show a correlation with chitotriosidase. Serum ferritin levels were also not correlated with body mass index (BMI).

Four GD1 patients had a history of repeated blood transfusions, exceeding 40 units in total. These patients had significantly higher serum ferritin levels (median 1122 µg/L (range 572-1520)) and hepcidin levels (median 9.4 nM (range 6.2-20.3)) as compared to non-transfused GD patients (median serum ferritin 304 (range 176-1306), p=0.008, median hepcidin level (4.0 nM (range 0.5-14.6), p=0.04).

Patients were stratified according to the presence or absence of persistent hyperferritinemia despite therapy (ERT/SRT). Results of this analysis are shown in table 3. Untreated patients (n=2) and patients on therapy for less than two years (n=4) were excluded from this analysis. Differences in iron profile, hepcidin and chitotriosidase levels between these groups were examined. Patients with persistent hyperferritinemia have significantly higher hepcidin levels and lower serum transferrin than patients with normal ferritin levels. Also, liver iron content as measured by MRI was higher in patients with persistent elevated ferritin levels as compared to patients with normal serum ferritin levels. Chitotriosidase enzyme activity was not different between the hyperferritinemic and non-hyperferritinemic patients.

Table 1. Characteristics of participants

	GD1 patients, n = 40	**Healthy controls, n = 40**
Age, years	50.5 (23-76)	51 (22-75)
Male:female	26 (65%):14 (35%)	26 (65%):14 (35%)
BMI, kg/m^2	24.3 (17.1-31.7)	24.4 (17.7-33)
Obesity (BMI>30 kg/m^2)	3 (7.5%)	5 (12.5%)
Splenectomy	10 (25%)	
On treatment	38 (95%)	
ERT:SRT	35:3	
Years on treatment	15 (0-24)	
Chitotriosidase, nmol/ml/hr	3,135 (324-23,192)	
Platelet count, x10^9/L	184 (58-364)	
Thrombocytopenia (platelets < 150 x109/L)	15 (37.5%)	
GBA1 genotype		
- N370S/L444P	14 (35%)	
- N370S/R120W	3 (7.5%)	
- N370S/N370S	2 (5%)	
- other / unknown	21 (52.5%)	

Continuous data are noted in medians and ranges (min-max). Categorical data are noted as number and percentage.
Abbreviations: BMI; body mass index.

Table 2. Haematological parameters and iron status

	Reference range	GD1 patients n=40	Healthy controls n=40	p-value
Haemoglobin (g/dL)	Men: 13.6-16.8 Women: 12.0-16.0	13.9 (11.0-16.3)	14.6 (11.7-17.3)	0.04
Mean corpuscular volume (fL)	80-100	90 (81-101)	88 (77-97)	ns
Ferritin (µg/L)	Men: 25-300 Women: 20-250	333 (40-1520)	152 (10-612)	<0.0001
Iron (µmol/L)	Men: 11-32 Women: 11-27	16 .0(5.4-30.4)	19.1 (5.9-30.1)	ns
Transferrin (g/L)	2.0-3.6	2.35 (1.7-3.69)	2.54 (1.68-3.68)	0.01
TSAT (%)	20-55	26 (9-55)	30 (6-54)	ns
Hepcidin (nM)	Men: <0.5-14.7 pre-menopausal women: < 0.5-12.3 post-menopausal women: <0.5-15.6	4.3 (0.5 – 20.3)	5.05 (0.5 – 15.3)	ns
[Hepcidin/ferritin] ratio (pmol/µg)	Men: 2.9-87.9 pre-menopausal women: 3-167.3 post-menopausal women: 9.1-143.1	12.9 (1.0-42.7)	33.6 (4.8-83.7)	<0.0001
[TSAT/hepcidin] ratio (%/nM)	Men: 1.7-256.3 pre-menopausal women: 2.0-330 post-menopausal women: 1.5-73.4	6.6 (1.3-61.7)	5.5 (1.3-46.7)	ns
Liver iron R2* (Hz)	< 51	41 (28-165)	38 (28-53)	0.006

Data are presented in medians and ranges (min-max). Reference ranges are according the local laboratory in the Academic Medical Center in Amsterdam. Reference ranges for hepcidin were obtained from hepcidinanalysis.com (visited April 2nd 2018) Testing was performed using paired t-test for normally distributed data (hemoglobin, MCV, iron, TSAT) and Wilcoxon-signed rank test for not-normally distributed parameters (ferritin, transferrin, hepcidin, hepcidin/ferritin ratio, TSAT/hepcidin ratio). P-values are provided for significant findings ($p < 0.05$). ns; non-significant

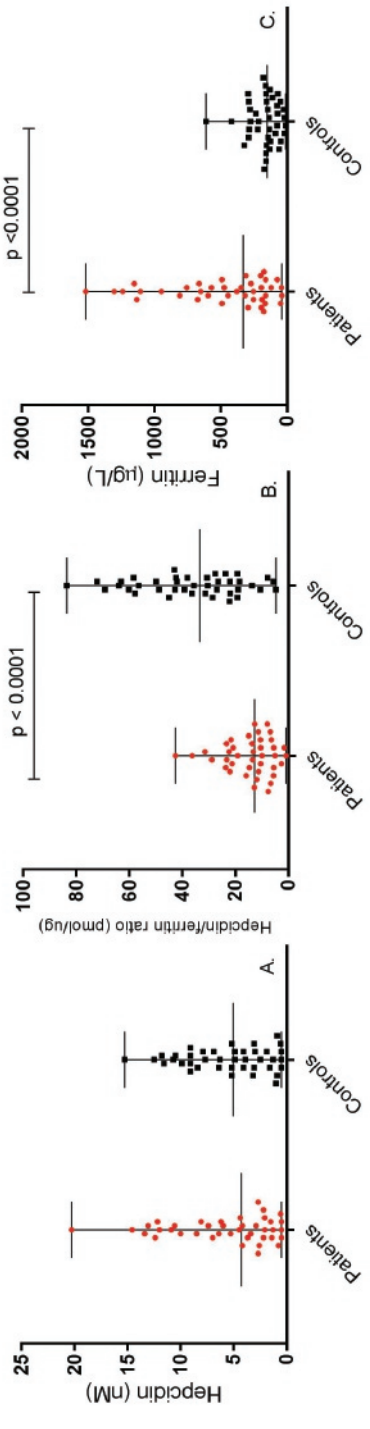

Figure 1. Hepcidin levels (A), hepcidin/ferritin ratio (B) and ferritin levels (C) in all GD1 patients and matched healthy controls. The horizontal bars indicate medians and full ranges.

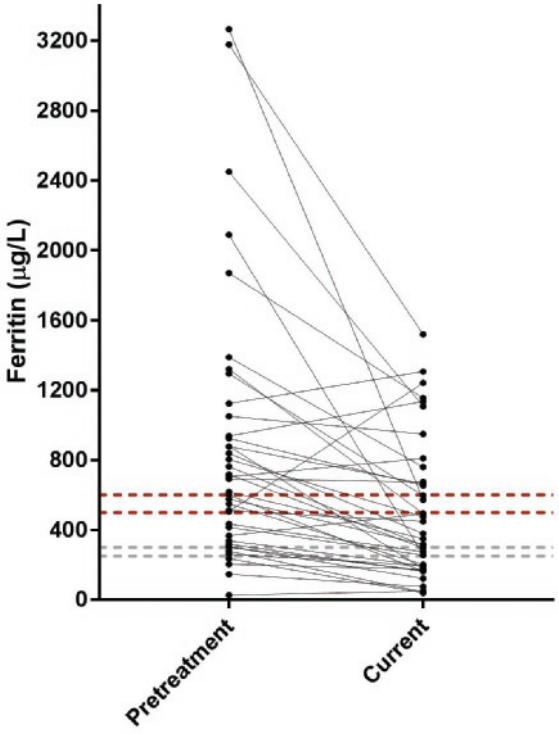

Figure 2. Course of serum ferritin levels for individual patients from pretreatment (before start of ERT or SRT) to current ferritin level. The dashed grey line indicates the upper limit of the normal reference range of serum ferritin levels for males (upper grey line, 300 µg/L) and females (lower grey line, 250 µg/L). The dashed red line indicates 2 x the upper limit of normal for the reference range (males upper red line, females lower red line).

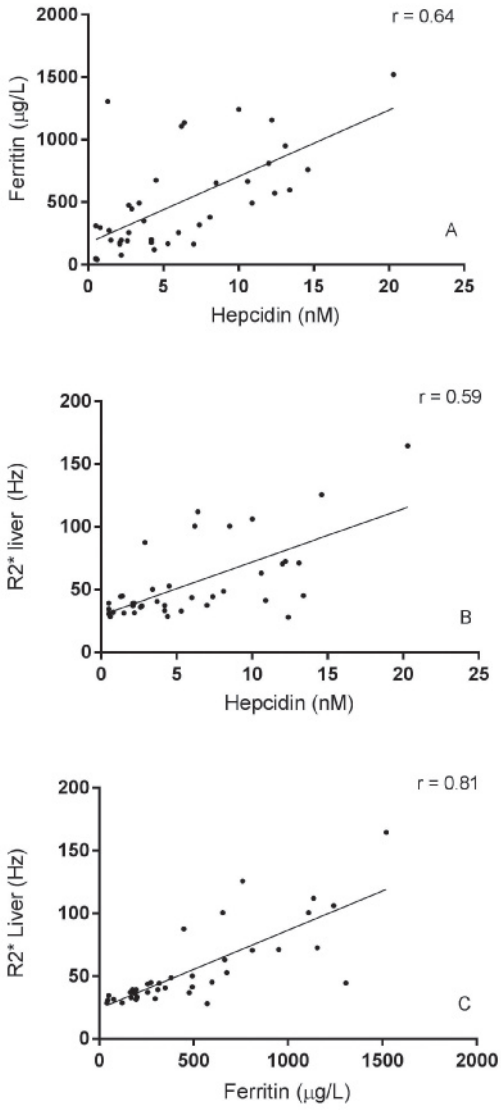

Figure 3. Correlations between hepcidin and ferritin levels (A) , hepcidin and liver R2* values (B) and ferritin and liver R2* values (C) in GD1 patients. r = Spearman's rho

Table 3. Comparison of patients with persistent hyperferritinemia despite therapy versus non-hyperferritinemic patients

	Patients with hyperferritinemia	Patients without hyperferritinimia	p-value*
No. of patients	18	16	
Years on treatment	16.5 (2-22)	18 (3-24)	0.35
Hepcidin (nM)	7.8 (0.5-20.3)	2.4 (0.5-7)	**0.0004**
Haemoglobin (g/dL)	13.8 (11-15.8)	13.9 (12.5-15.5)	0.87
Iron (µmol/L)	16.4 (9.5-30.4)	15.7 (5.4-21.4)	0.52
Transferrin (g/L)	2.1 (1.7-3.5)	2.4 (1.8-3.7)	**0.04**
Tsat (%)	27 (17-55)	24.5 (9-40)	0.11
Liver iron R2* (Hz)	67 (28-165)	34.5 (29-44)	**<0.0001**
Chitotriosidase**	3454 (438-11358)	2278 (324-23192)	0.48

Hyperferritinemia is defined as serum ferritin levels >300 µg/L for males and >250 µg/L for females. Data are given in medians and ranges. Untreated patients and patients on therapy for less than two years were excluded from this analysis. *Mann-Whitney U testing was applied. ** Two patients with deficient chitotriosidase activity (one in the hyperferritinemic group and one without hyperferritinemia) were excluded from this analysis.

Discussion

In this study we reported no difference in absolute levels of hepcidin, the key regulator hormone of iron homeostasis, when comparing GD1 patients to matched healthy controls. Serum ferritin levels were elevated in the majority of patients before start of ERT or SRT and persisting hyperferritinemia despite therapy for more than two years was found in 53% of the studied population. As a result, the hepcidin/ferritin ratio was significantly lower in GD1 patients. This points at an inappropriate hepcidin response to iron loading. In other words, the hepcidin level is too low for the circulating ferritin levels. A lowered hepcidin/ferritin ratio is also seen in hereditary haemochromatosis (HH) [35]. A mutation in the *HFE* gene in HH leads to hepcidin deficiency. The key difference between HH and GD is that in HFE-HH, the hepatocyte signaling of circulating and stored iron to hepcidin synthesis is impaired and consequently, circulating iron (TSAT) and parenchymal iron are elevated [44]. However, in GD, through an incompletely understood mechanism, TSAT is normal and the iron is mainly sequestered in the RES. It thus appears that both impaired function of the *HFE* gene in HH as well as an iron distribution disorder in GD result in inappropriately low hepcidin levels for ferritin levels.

Evidence of iron loading in the current study was shown by elevated R2* levels on MRI examinations of the liver. The liver R2* values correlated with both hepcidin- and ferritin levels. Also, hepcidin and ferritin levels did show a correlation. Indeed, patients with persistent hyperferritinemia despite therapy for GD were shown to have higher hepcidin levels as compared to patients with normalized ferritin levels after > 2 years of ERT

or SRT. This finding reflects the existence of a certain degree of hepcidin response to iron loading. The feedback mechanism seems partially intact, as hepcidin is higher in response to elevated ferritin levels in this hyperferritinemic subgroup as compared to non-hyperferritinemic GD patients.

Interestingly, chitotriosidase levels did not differ between patients with and without persistent hyperferritinemia. Chitotriosidase, a well-established GD biomarker is used as a reflection of total burden of Gaucher cells [30]. Clearly, the presence of residual GD as measured by chitotriosidase, is not predictive of the presence of a risk of iron overload. This finding indicates that serum ferritin levels may be of higher importance than residual Gaucher cell load per se in predicting the risk of late complications in a GD patient. Increased iron storage can lead to cellular toxicity with a subsequent risk of complications, for example fibrosis and malignancy [45-47]. Therefore, it is likely that hyperferritinemic patients are at increased risk for developing these complications irrespective of their chitotriosidase levels.

Although an earlier study reported high hepcidin levels [36], more detailed recent investigations confirm our findings of a discrepancy between ferritin and hepcidin levels [27,37]. In the study by Lorenz et al, all 11 GD patients were untreated in contrast to our population with 95% of patients being treated with ERT or SRT. The report by Lefebvre et al. confirmed normal hepcidin levels in a large cohort of 90 GD1 of whom 34 were untreated. In response to ERT, studied in 10 patients, anemia improved and TSAT increased, suggesting the release of stored iron from Gaucher cells. Interestingly, hepcidin levels appeared to increase transiently over time, suggesting a temporary “defense” against the release of sequestered iron in the circulation [37]. A limitation of the current study is the fact that we mainly included patients on treatment. This possible effect of treatment on hepcidin levels is shown to be limited, as normal hepcidin levels were also found in non-treated GD patients [37].

It is intriguing why the hepcidin/ferritin ratio is altered in GD. Hepcidin is important in maintaining iron homeostasis in the human body. The expression of this hormone is regulated by several mechanisms, of which iron availability and stores, erythropoietic drive, hypoxia and inflammation are the main determinants [32]. In states of inflammation, an increase in hepcidin leads to downregulation of ferroportin expression. This in turn leads to less iron absorption from the gut and iron sequestration in macrophages of liver and spleen. Consequently, less iron is transported to the extracellular fluid and invading

extracellular pathogens are depleted from iron, which is necessary for their survival [48]. The clinical picture of anemia of inflammation can arise in this setting [21,23]. It is thought that in GD, the chronic inflammatory state could contribute to a disturbed iron metabolism. However, there is evidence for a pro- as well as anti-inflammatory state in Gaucher disease [27,49-51]. For example, Lorenz showed mild elevations of TNF-alfa in some patients, which decrease upon treatment [27]. We have previously postulated that individual differences in balance between pro- and anti-inflammatory states may impact on body iron distribution [31]. However, the current study shows that upregulation of hepcidin-expression as a cause of altered iron distribution is absent. In the study by Lefebvre et al it was suggested that hyperferritinemia in GD exists independent of systemic inflammation as all participants in this study were negative for C-reactive protein (CRP) and interleukin6 (IL-6). Of interest in this respect is their observation that ferroportin membrane expression is decreased in their in vitro macrophage Gaucher cell model. However, this model clearly shows an inflammatory response to the induction of storage by conditurol B epoxide (CBE), which contradicts the finding of absence of inflammation markers in their patients [37].

Bringing all evidence together, we hypothesize that hepcidin upregulation is insufficient in GD. This leaves room for other factors than systemic inflammation to impact on hepcidin secretion. One of those factors is ineffective or increased erythropoiesis, in which several erythroid ligands are proposed to influence hepcidin-levels. Growth differentiation factor 15 (GDF15) and erythroferrone (ERFE) are secreted by erythroblasts in case of ineffective or increased erythropoiesis and are shown to have a hepcidin suppressive effect [52,53]. In GD, hypersplenism leads to increased rates of breakdown of red blood cells and bone marrow infiltration by Gaucher cells may induce impaired erythropoiesis, which results in anaemia [54]. Also, morphological changes in red blood cells have been described and may favor increased erythrophagocytosis [55,56]. Those pathological effects on erythropoiesis might also have an hepcidin-suppressive effect as is shown in thalassemia syndromes [57,58]. In contrast, the need for regular blood transfusions in beta-thalassemia major results in relatively higher hepcidin levels as transfusion leads to iron supply, decreasing the erythropoietic drive [59]. In our cohort of GD patients, four patients had a history of multiple blood transfusions exceeding 40 units in total. These patients showed higher hepcidin levels compared to patients without a history of regular transfusions.

In conclusion, we found no differences in hepcidin levels in GD1 patients as compared to controls. The lowered hepcidin/ferritin-ratio indicates an inappropriate response of hepcidin to iron loading. Hepcidin expression in the studied cohort is likely to be influenced

by several counteracting factors. We speculate that the sum of the main contributing factors; inflammation, iron stores and distribution, increased or ineffective erythropoiesis, local and systemic effects of Gaucher cells, determines the presence and degree of iron loading. Chitotriosidase activity does not necessarily correspond to the degree of iron loading as measured by elevated ferritin levels. In GD, excess iron is mainly stored in macrophages, as TSAT are within normal range in most patients. Even after years of treatment, residual iron storage may remain, which not only reflects residual disease, but also carries a risk for late complications.

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