Ironing out pathophysiological aspects of Gaucher disease

Regenboog, M.

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General Discussion
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The development and implementation of highly effective therapy for GD has altered the lives of many patients. Enzyme replacement therapy with imiglucerase (Cerezyme®, Genzyme-Sanofi, Cambridge, MA, USA) and velaglucerase alfa (Vpriv®, Shire, Lexington, MA, USA) as well as the more recently authorized oral substrate reduction therapy eliglustat (Cerdelga®, Genzyme-Sanofi, Cambridge, MA, USA) all result in alleviation of clinical signs and symptoms, reduced biomarker levels indicating decreased Gaucher cell burden, together with improvement in quality of life [1,2]. However, not all patients reach a state with resolution of symptoms and low biomarkers. The presence of residual disease in these patients might contribute to the development of late complications and associated conditions. Presumably, timing of treatment initiation is of utmost importance [3-5].

Over the last decades, more and more attention has been paid to the occurrence of associated conditions and late complications in GD, including Parkinson disease and malignancies. The importance of prevention or early detection of these often life-threatening conditions is indisputable. A growing body of evidence is available regarding the factors possible contributing to the pathophysiological changes as seen in GD and its morbidities. One of those intriguing changes is the altered iron distribution, the subject of this thesis. The clinical meaning, causes and consequences of this finding will be discussed further below. By studying this piece of the large and complex pathophysiological puzzle of GD, the aim of this thesis is to improve our understanding of this rare lysosomal storage disorder. This is necessary to improve clinical-decision making and risk-assessment in each individual GD patient.

Gaucher disease related conditions: a clinical perspective

Only few studies have systematically addressed the development of complications or associated conditions and the risk factors involved. In table 1 a summary of risk factors is provided for the most well-known of these conditions as encountered in GD.
Skeletal complications such as avascular necrosis, bone infarcts and pathological fractures are severe complications of GD [6,7]. Prior to the introduction of ERT, patients suffered from longstanding high burden of Gaucher and subsequent pathophysiological changes. This “delay” in treatment initiation results in an increased risk of developing bone complications. Another risk factor for bone complications is a history of splenectomy. Splenectomy might be an indicator of severe disease by itself, but absence of the spleen will also promote further storage in other sites, such as the liver and bone marrow [8,9]. Hence it is likely that splenectomy supports the emergence of bone disease. Not only bone disease is related to splenectomy: pulmonary complications and severe liver involvement are all associated with a history of removal of the spleen in GD [10,11].

Pulmonary complications, specifically pulmonary hypertension as complication of GD is rare but may be severe and life threatening [12]. Besides a history of splenectomy, other predisposing factors to develop pulmonary hypertension were reported to be female sex and polymorphisms in the ACE gene [10]. A potential pathophysiological process is macrophage dysfunction, which can induce vascular changes in the pulmonary system leading to pulmonary hypertension. Also, hepatopulmonary syndrome as a consequence of liver cirrhosis can contribute to the development of this complication [13].
Liver involvement encompasses more than just hepatomegaly. Although in most patient liver function is well preserved, liver fibrosis and cirrhosis can develop [14,15]. In relation to this, cases of portal hypertension and oesophageal varices have been documented [15,16]. It is thought that after splenectomy, increased liver involvement contributes to the risk of hepatic complications. With the development of fibrotic and subsequent cirrhotic changes, the risk for occurrence of hepatocellular carcinoma (HCC) also increases [17]. Some case reports of HCC in GD patients were published in the past [18-20]. All three patients in these reports had a history of splenectomy and two were also diagnosed with concomitant hepatitis B infection, as potential risk factors. Chapter 5 further elaborates on this and other risk factors, which are further discussed below (see section “The risk for HCC development: towards improved screening strategy”).

Next to HCC, the increased incidence rates of other cancers, specifically of haematological origin such as multiple myeloma (MM) and non-Hodgkin lymphoma (NHL), are described in the GD population [17,21-23]. The frequent finding of gammopathies (monoclonal or polyclonal) in GD patients is a preceding hallmark that predicts the increased incidence rates of these malignancies [24]. Once a GD patient develops a monoclonal gammopathy, it remains present despite administration of ERT [25]. However, it was also shown that ERT is able to decrease or stabilize immunoglobulin levels in patients with and without monoclonal gammopathy [24]. None of the studied patients in this cohort developed MGUS or MM whilst on treatment with ERT [4]. Probably, as is the case for several other associated conditions and complications in GD, again, timing of treatment initiation is important in preventing the development of MGUS.

Lastly, the association between GD and Parkinson disease is important to mention. Even among carriers of a GBA1 mutation, the risk of developing Parkinson disease is increased as compared to the general population [26]. It was shown that Parkinson disease in a GD patient has an earlier onset than sporadic Parkinson disease in general [27,28]. However, at present, no clear risk factors for the development of Parkinson, other than a GBA mutation, in a GD patient are known. The mechanisms causing neurodegeneration in GBA1-mutation associated Parkinson disease are subject of research. A link between lowered glucocerebrosidase activity and elevated levels of α-synuclein that can cause the formation of Lewy-bodies is described [29]. The elucidation of the precise pathogenic mechanisms eliciting Parkinson disease in GD is subject of research [30,31].
Several other conditions related to GD or its treatment such as peripheral neuropathy, gallstones, metabolic syndrome, peripheral insulin resistance and hepatic steatosis have all been described, but will not be further discussed here [32-36].

**Pathophysiological aspects; ironing out the role of iron**

The pathophysiology of GD is complex. Clinical symptoms and risks for complications and associated conditions are likely the result of genetic, epigenetic and environmental factors, such as infections. The influence of epigenetic or environmental factors is illustrated by the extremely heterogeneous phenotypic expression in families carrying the same genotype with the most striking being discordant phenotypes in monozygotic twins [37,38]. The complexity is also illustrated by the lack of a clear integration of different abnormalities that have been studied and that have been suggested to play a role in pathophysiology. The pathological hallmark of GD is the presence of Gaucher cells loaded with glucocerebroside. These typical macrophages are thought to induce local effects in the main storage sites. Disturbed tissue architecture might directly affect proper functioning of the organ involved, and also lead to areas of infarction as a result of impaired blood flow. Next to the local effects of glucocerebroside accumulation in Gaucher cells, several systemic abnormalities are proposed to arise. Inflammatory effects with release of pro- and anti-inflammatory cytokines, immunological abnormalities and pathological cellular changes are all key aspects involved. Increased plasma levels of the potentially toxic by-products of glucocerebroside storage, such as glucosylsphingosine (GlcSph) and globotriaosylsphingosine (lysoGb3), which may play a role in the inflammatory state in GD have been described [39]. Different studies have suggested a prominent role for GlcSph in the development of GD-related complications, such as bone complications and B-cell proliferation [40,41]. Another factor that may also exhibit toxic effects and as such contributes to pathophysiological changes in GD is iron, of which the precise mechanism is still unknown.

This thesis aims to answer questions regarding the role of iron in the pathophysiology of GD. It is important to realize that this is a part of the bigger picture and other pathophysiological mechanisms, as briefly addressed above, are likely to be all involved to different extent in each individual patient. The reason to further elucidate a potential link of iron metabolism to pathophysiology of GD stems from early reports from the 1960s and afterwards, in which iron particles in Gaucher cells were described [42,43]. These studies, together with later observations of extremely elevated ferritin levels, led to hypotheses about iron as disease modifying factor in GD [44-46].
Iron is an essential element in the human body. It is important in haemoglobin production for erythropoiesis, but also needed for fundamental metabolic functioning. Iron is obtained from the diet, in heme and non-heme forms. As excessive levels of iron are toxic, homeostasis is tightly regulated by systemic and cellular regulatory processes. After entering the circulation via transport through duodenal enterocytes, iron is bound to transferrin; a glycoprotein that is capable of binding two iron-atoms and distributes iron throughout the body. Plasma iron bound to transferrin is directed to sites where it is needed, such as the bone marrow for red blood cell production, or to storage sites where iron can be saved for later use. Ferritin is the main cellular iron storage protein that stores iron in a non-toxic form, predominantly in hepatocytes and macrophages. The liver-derived hormone hepcidin regulates iron release from storage sites and uptake from dietary sources by inhibiting the cellular iron exporter ferroportin [47,48].

In GD, several studies have shown that extremely elevated ferritin levels are present and suggestions of a disturbed iron homeostasis are made [45,46,49-51]. For the first time, the current studies in this thesis have shown the locations of iron storage in GD patients, i.e. mainly the liver and bone marrow and not the spleen or other organs. Serum ferritin levels are shown to reflect increased iron levels as visualized by using magnetic resonance imaging (MRI) in chapter 3. The causes and consequences of this altered iron levels will be discussed below.

**Causes and consequences of an altered iron distribution in GD**

In chapter 4 we have shown that hepcidin levels remained within normal ranges, excluding the possibility of hepcidin upregulation in our patients as a cause of increased iron storage. As hypothesized in chapter 2, the polarization of macrophage subtypes can influence the iron storage pattern. The classically-activated, pro-inflammatory, cell types with increased hepcidin expression leading to macrophage iron sequestration versus the alternatively-activated macrophages which are associated with a negative regulation of hepcidin production and subsequent iron efflux from storage sites, have influence on the individual pattern of iron storage in patients. Hence, the balance in the different types of macrophages is likely to be of importance in a patient’s predisposition to iron loading. In addition to this phenomenon, clues towards an insufficient response of hepcidin to iron sequestration are presented in chapter 4. GD patients were shown to have a significantly lower hepcidin-ferritin ratio as compared to healthy controls. Under normal circumstances, increased
storage of iron results in increasing hepcidin levels to protect the human body for further uptake and circulation of free iron. Explanations for the relative hepcidin suppression in GD could be attributed to a similar effect as is seen in cases of ineffective or increased erythropoiesis, as is also known to occur in thalassaemia syndromes [52]. However, it still remains unclear whether hepcidin dysregulation is the primary cause of increased iron storage or whether iron is sequestered in macrophages irrespective of hepcidin levels. The diseased Gaucher macrophages are cells of which membrane properties are affected by the sphingolipid accumulation. As such, normal functioning of these cells could be impaired [53]. Perhaps the sensing of elevated iron stores is also affected, leading to an absence of hepcidin response to iron loading. Besides, it is presently unknown whether iron in Gaucher cells is able to exit those cells. The expression of ferroportin on the membrane of macrophages in GD could be affected as well. As a result, iron particles might be trapped in those cells. A clue pointing towards such a phenomenon was described in a recently published study [51], in which ferroportin membrane expression in a Gaucher cell model was decreased. However, this pro-inflammatory model did not correspond with the findings in patients, where an inflammatory response was absent.

The assumption that iron is mainly sequestered in macrophages is based on the normal transferrin saturations (TSAT) found in our studied cohort. This is in contrast to the iron-loading as found in iron overload syndromes such as hereditary haemochromatosis, in which increased TSAT and excessive iron in parenchymal cells are found [54]. However, it is not unlikely that increased iron stores in a GD patient might also shift towards parenchymal storage in response to treatment. In fact, in three cases of which data on TSAT was available as described in chapter 5, elevated TSAT-levels were found and correlated with positive iron-staining in macrophages as well as hepatocytes in liver tissue on histopathological examination. The presence of excessive iron levels is associated with a risk of carcinogenesis [55]. When iron circulates in free form (non-transferrin bound) oxidants can be formed as a result of the easy interconversion of Fe^{2+} to Fe^{3+}. This formed reactive oxygen species have a direct toxic effect on proteins and DNA with subsequent organ damage and a risk of developing malignancies. Even longstanding exposure to low levels of excess iron can lead to toxicity [56]. As was also described in chapter 5, iron toxicity is considered as contributing factor to the increased risk of HCC in GD patients. Therefore, monitoring iron status in this population is necessary, next to assessing other risk factors which will be discussed below.
The risk for HCC development: towards improved screening strategy

As was shown in chapter 6, focal lesions in liver and spleen are common in GD. In this retrospective study, 24% of the studied GD1 patients had a lesion in the liver. As benign focal hepatic lesions, such as gaucheroma or focal nodular hyperplasia, are sometimes hard to distinguish from a possible malignant lesion based on imaging characteristics alone, it is important to closely monitor those anomalies. When a radiologist is able to define a clear diagnosis of a benign focal lesion, such as a simple cyst of haemangioma, no follow-up is required. However, any doubt regarding an (imaging) diagnosis should warrant close monitoring, as was also proposed in the follow-up algorithm in chapter 6. In particular, as GD patients are at an increased risk for HCC development, signs of a possible HCC should not be misinterpreted as a benign focal lesion.

Currently, no clear-cut evidence-based screening strategy for HCC detection in GD patients is available. Based on the findings in the case series described in chapter 5 a practical recommendation for screening for GD patients with one or more of the following risk factors was proposed:

1) History of splenectomy. 2) Presence of liver fibrosis/cirrhosis. 3) Persistent hyperferritinemia in combination with TSAT >45%. 4) Chronic hepatitis B/C carriers.

Previously, at our centre, the policy was adopted to screen all splenectomized patients every six months by ultrasound examinations. So far this has only revealed one HCC during ten years of follow-up in around twenty patients. This patient also had a very high ferritin level and TSAT and is part of the series described in this thesis.

Patients who had undergone splenectomy in the past were considered at risk for hepatic carcinogenesis as splenectomy could lead to advanced liver involvement of GD. In this light, the finding that also patients with an intact spleen did develop HCC, was surprising. Three patients with an intact spleen developed liver cirrhosis. As liver cirrhosis is the main underlying cause of HCC in the general population, the presence of cirrhosis in a GD patient also warrants close monitoring. As liver cirrhosis is irreversible, it is desirable to detect its preceding histological hallmark of liver fibrosis. Liver biopsy is the reference standard in diagnosing liver fibrosis, but other less invasive procedures to determine liver stiffness are available. Although not validated for use in GD, quantitative imaging techniques such as magnetic resonance elastography (MRE) and transient elastography (TE; Fibroscan®) have proven to be accurate in detection of liver fibrosis in other chronic
liver diseases [57]. In a small cohort of GD patients the use of MRE and TE was analysed and proposed as techniques to identify patients with liver fibrosis [11]. Higher liver stiffness values were reported for splenectomized patients. A main drawback of MRE was the fact that this method was not feasible in some patients due to high iron load of the liver, making TE more suitable in this population. A suspicion of excess iron storage should be raised when a GD patient shows persistent elevated ferritin levels. As described above, this finding is shown to be correlated to signs of iron storage on MRI, which is likely to occur in the liver. However, the cellular storage site is likely to vary in each individual. Although a lot of data is missing on iron-staining in histopathological examinations of liver tissues in the cases in chapter 5, we showed that three patients with increased TSAT (>45%) all showed positive iron-stains with iron in macrophages as well as hepatocytes, which clearly contributes to the development of HCC. As is known from other iron-overload disorders, increasing TSAT reflect increasing levels of non-transferrin bound iron (NTBI) with a subsequent risk of parenchymal iron storage and damage. MRI can be used to screen for iron overload, but the cellular storage site of iron cannot be determined based on MRI. Therefore, elevation of TSAT in a GD patient with hyperferritinemia should warrant screening for HCC. Chronic hepatitis B/C carriers have an increased risk for HCC development, also when liver cirrhosis is absent [58]. The presence of these comorbidities in a GD patient is therefore a reason for screening for HCC.

Based on these findings one could also argue in favour of narrowing down the proposed screening strategy as follows:

Screening for HCC in a GD patient should be carried out in every patient with one of the following risk factors:

1) Presence of liver fibrosis/cirrhosis. 2) Persistent hyperferritinemia and TSAT >45%. 3) Chronic hepatitis B/C carriers.

In splenectomized GD patients one should always be aware of advanced hepatic involvement, but when the abovementioned risk factors are absent in a patient with a history of splenectomy, it is reasonable to discontinue HCC-screening. The presence of liver fibrosis or cirrhosis can be detected by non-invasive elastography methods. As iron deposition can induce failure of MR elastography measurements, the use of TE in GD can be advocated as first choice for liver stiffness assessment. However, the use of the currently available quantitative imaging techniques has not been validated in GD. Also,
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it remains to be clarified which stage of fibrosis should be used as cut-off for entry in the HCC surveillance program. It seems appropriate to define significant fibrosis (METAVIR scoring system F≥2 [59]) in a GD patient as a risk factor for HCC development. Further research should be carried out to evaluate the (cost) effectiveness of this approach.

**From storage to associated conditions: monitoring residual disease**

One common risk factor for several complications and associated conditions seems the timing of intervention. GD patients diagnosed prior to the introduction of ERT have been untreated for years, with a subsequent higher GD burden. Nowadays, a substantial number of GD patients have signs of residual disease, and as discussed previously, monitoring of residual disease is thus important for prevention - or early detection - of those conditions.

GD is a multi-compartmental disease with several different sites involved to a different degree in each individual patient. Over the years, several biomarkers have been established to aid the clinician in daily care and monitoring of GD patients. As we have shown in chapter 3 and 4, serum ferritin levels are important in follow-up of GD as they reflect excess levels of iron storage. We postulated that the storage of iron is associated with the presence of residual disease. By using iron-sensitive MRI protocols, areas with excess iron in the body could be precisely localized and quantified. However, chitotriosidase levels did not correlate with the MRI-measured iron levels. High levels of chitotriosidase reflect a high burden of Gaucher cells, but not necessarily an increased storage of iron in these cells. Different types of Gaucher macrophages are shown to exist, and as such the iron storage pattern might also vary. Indeed, in our MRI study the splenic tissue of GD patients is relatively free of iron, whereas the spleen is one of the main sites involved in Gaucher cell storage as is also reflected by high chitotriosidase levels in several patients. As such, the presence of increased iron storage reflects residual disease in GD but the absence of iron storage does not exclude the presence of residual disease. Therefore, the studied whole-body iron-sensitive MRI protocol is not applicable in overall quantification of residual disease activity, but rather a tool to assess the risk of iron-related complications.

As we have shown that iron deposition only occurs in liver, spleen and bone marrow in GD patients, it seems practical to monitor these sites for iron. A monitoring approach for the future could include a baseline iron level assessment for every GD patient, with frequent analysis of iron status (ferritin levels, total iron and transferrin saturation). In case of persistent abnormalities in iron status, i.e. after two years of adequate treatment, an additional MRI-scan to detect sites of increased iron storage as compared to baseline could reveal a patient at risk for iron-related complications. Not only the increased risk
for malignancies, as discussed earlier, is associated with iron toxicity. An excess of iron in the brain is also implicated as contributor to the pathogenesis of Parkinson disease [60] and may induce insulin resistance [61].

**Future perspectives**

Ever since the introduction of treatment for GD, clinical care for GD patients has improved. Treatment early in the disease course can help prevent further storage and cell damage. Although systematic studies in larger cohorts are lacking, it is likely that complications are avoided by timely initiation of therapy. As discussed, no MM case has occurred in the Dutch cohort in patients who had no MGUS before ERT initiation. In addition, no HCC has arisen in a patient without a longstanding history of GD. Ideally, a large prospective study on the occurrence of malignancies should be carried out. In this way, specific risk factors, pathophysiological changes and characteristics of the GD patient developing cancer could be studied in more detail.

Based on the findings in chapter 5 we recommend a surveillance program for HCC, as outlined above (section “The risk for HCC development: towards improved screening strategy”). As already mentioned, further research should be carried out to evaluate this approach. In particular the applicability of TE in this population should be assessed. Ideally, a validation study of this method in GD should be performed.

With regard to changes in iron metabolism and storage in GD patients, it could be valuable to study patients with signs of iron loading (increasing TSATs) and a possible beneficial effect of chelation- or venesection therapy. This is only described in a small study [49]. However, it is questionable whether the excess of iron stored could be released from the storage cell in response to this kind of therapy. As was also suggested in chapter 4, iron trafficking from the Gaucher macrophages could be affected due to changes in ferroportin-expression on the membrane of these cells. Further research regarding cellular changes accompanying the changes in iron distribution in GD should be carried out. This should include the measurements of reactive oxygen species (ROS) in Gaucher cells. It is rational to speculate on the development of ROS as a result of iron loading in macrophages. If these damaging ROS are present in the typical Gaucher cells this could well be the result of increased iron levels and as such be identified as risk factor for cellular damage and malignant transformation. In conclusion, further studies will need to be carried out to understand the effect of iron trapping in Gaucher disease. To end with a positive message: the prospect for GD patients is bright, given the many treatment options and
available diagnostic modalities to prevent irreversible disease and potentially the life-threatening long-term complications and associated conditions. This thesis contributes to the continuously expanding amount of knowledge regarding several pathophysiological aspects of the disease, and as such, to the improvement of monitoring of (residual) disease.
References


