Gaucher disease type I: associated morbidities and long term efficacy of enzyme replacement therapy

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Discussion
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Section I. Enzyme replacement therapy
The success of enzyme replacement therapy for Gaucher disease type I starts with the first study of mannose-terminated glucocerebrosidase (Genzyme Corp., Mass. USA) that was carried out in 12 patients. A relatively large intravenous dose of 60 units/kg every two weeks (10 patients) or weekly (2 patients) was given. Striking and rapid improvement in clinical parameters led to prompt approval and marketing of the enzyme. Since then, more than 3000 patients worldwide are treated with enzyme replacement therapy. However, no matter how successful, life-long weekly or two-weekly intravenous therapy is burdensome. Lowering the frequency of infusion could improve quality of life but had only been studied in patients that had not yet reached stable disease parameters, and proved unsuccessful in this group. The international consensus statement for maintenance therapy has no recommendations concerning frequency of administration. Our study now shows that maintenance therapy in most adult patients is feasible using a once every 4 weeks schedule at the same total monthly dose. Some patients will not be suitable for low frequency of administration and it seems that especially patients with a relatively low baseline bone marrow fat fraction, as an indicator that Gaucher cells still reside in this compartment, are at risk. This makes close monitoring of all disease parameters for early detection of disease progression and adequate frequency adjustment imperative.

Alternatives for more convenience for patients can be provided by home treatment, which has been proven to be safe and feasible. Oral treatment by substrate reduction (miglustat, Zavesca®, Actelion, Basel, Switzerland) is possible, but its use is limited to patients with mild to moderate disease who are unsuitable for enzyme replacement therapy. Early studies have shown favorable effects of maintaining disease stability after switching from enzyme replacement therapy to substrate reduction. A recently published trial in type I patients who either switched to substrate deprivation or continued their original regimen after a minimum of two years of enzyme therapy, also showed essentially stable disease in the study group. At present, a large multi-center trial is conducted to address the question as to which patients can tolerate to switch to substrate reduction and which patients cannot.

After the rapid approval of the enzyme based on the initial trial, the pharmaceutical company has not undertaken a multi center study with the aim to determine the lowest possible dose that resulted in an adequate clinical response. This issue is even more important in relation to the enormous costs of the enzyme, €55,000-300,000 per patient per year depending on dose and weight. A meta analysis of a number of independent studies did not show a significantly better response in high dose enzyme replacement therapy, although at low frequency of infusion (once every 2 weeks), the lowest dosages (≤20 U/kg/4wks) did seem inferior. However, patient numbers were small, and bone disease was not evaluated in a
quantitative manner. Guidelines for dosing protocols are based on consensus rather than on scientific evidence. In practice, the original regimen, using 60U/kg every 2 weeks, is prescribed by most physicians.

Our study now demonstrates that improvement of hemoglobin, platelet count, and liver- and spleen volume is not dose-dependent. However, chitotriosidase does respond better to a relatively high initial dose. Improvement in bone marrow burden (BMB), a MRI based scoring system, shows a strong trend towards a better response to higher doses, particularly in patients with more extensive bone marrow disease in whom the difference is statistically significant. Recently our results have been extended. A large retrospective study using data of 975 patients from the international Gaucher registry (ICGG) resulted in a dose response relationship for hematological parameters and organomegaly. Bone disease was not assessed. Recent data on the effect of enzyme replacement therapy on bone mineral density is discussed elsewhere in this chapter.

The slightly better response of hematological parameters and organomegaly to high dose enzyme replacement therapy when analyzed in large cohorts may in general not result in a relevant clinical difference that would justify the costs of high dosing. On the other hand, it has now become clear that in case of severe bone disease, as well as in life-threatening situations, such as severe cytopenia, bleeding or excessive splenomegaly, treatment with a high dose will probably lead to a more rapid response. After the patient has reached a less vulnerable situation, the dose can be tapered. The complete assessment of disease, including determination of bone marrow involvement in addition to hematological, visceral and biochemical parameters is mandatory for determining the moment and dose for the initiation of enzyme replacement therapy.

A returning issue in this thesis is the difficulty of adequately treating bone disease, at this moment the biggest challenge in the care for Gaucher disease patients. Bone disease in Gaucher type I is characterized by bone marrow infiltration of Gaucher cells as well as defective bone remodeling, leading to osteopenia, osteonecrosis, bone crisis, pathological fractures and avascular infarction. The pathology is incompletely understood, and cannot be explained solely by the mass effect of Gaucher cell infiltration in the bone marrow. Cytokines, locally released hydrolases and a disturbed balance between osteoblasts and osteoclasts are probably also involved.

Compared to the other Gaucher-affected compartments, bone and bone marrow respond much slower to enzyme replacement therapy and in some patients require high doses of enzyme. The bone compartment is extremely vulnerable, with a high risk of relapse due to drug holidays or lengthening of dose intervals. All this is complicated by the fact that adequate monitoring requires advanced radiological equipment, which is not readily available in many centers.
In this thesis we show that in a subset of patients with severe bone disease prior to start of therapy, increasing dosages of enzyme do not improve clinical bone disease. Patients at risk are those with previous bone complications, a large liver, low bodyweight and the absence of a spleen at baseline, as well as a slow improvement in QCSI (Quantitative chemical shift imaging), chitotriosidase and MIP-1β during enzyme replacement therapy. The lack of improvement cannot be explained solely by the irreversible damage due to past bone complications. In fact, large areas of Gaucher cells continue to exist in the bone marrow, somehow not responding to the exogenously administered enzyme.

Why do these Gaucher cells survive the effects of the administered enzyme? The most probable explanation is that the enzyme is not targeted to the Gaucher cells due to altered properties of these cells. In the past, scarcely any beta-glucosidase activity could be found in marrow samples of patients that were treated with (high dose) enzyme replacement therapy. Possibly, various sub-populations of Gaucher cells exist, that differ in their ability to take up the exogenously administered enzyme. This was suggested from a study in patients with longstanding Gaucher disease, in whom splenic Gaucher cells had no detectable expression of mannose receptors.

The theory that a subset of patients is beyond responding is not new. Beutler hypothesized that if it is true that some patients require a high dose while others respond adequately to a low dose, one would expect the number of treatment failures to diminish when dose is increased following an individualized dosing protocol. This is not the case and so he concluded that a subset of patients for some reason does not respond properly.

What policy is appropriate for these patients? As stated previously, all patients with severe bone disease should be treated initially with high dose enzyme replacement therapy. Bone disease should preferably be assessed by MRI-based techniques, with quantification by BMB or QCSI. If long term high dose enzyme therapy does not result in amelioration of bone infiltration, one should question whether continuing the high dose is necessary and appropriate. Based on our data of a limited group of patients, we cannot recommend an exact period of time in which an increase in QCSI or a decrease in BMB can be expected on high dose therapy. Surely, this period should be long enough for the typically slow bone response to occur, although usually responses can be seen within 12-24 months. In the absence of a bone response, maintaining stable disease is the best we can achieve and dose could probably be tapered. In addition, supportive measures, such as bisphosphonates should be considered. Whether combination with substrate deprivation therapy has an additional positive effect on bone is currently unknown.

Recently a considerable body of data concerning bone mineral density in Gaucher disease has become available. Many patients exhibit signs and symptoms of osteopenia. Conflicting results with respect to the effect of enzyme replacement therapy on bone density have been described. Bisphosphonates improve bone mineral density in
combination with enzyme replacement therapy\textsuperscript{23}. However, caution should be taken when interpreting these results since a correlation between bone density and the risk of fractures in Gaucher disease type I has not been established. In addition, the use of DEXA may reveal false high or normal results in patients with sclerotic areas, avascular necrosis or collapsed vertebrae.

**Section II. Biomarkers**

Biomarkers are essential in clinical decision-making concerning diagnosis, determination of severity of disease and monitoring of therapeutic efficacy. In practice, the most widely used biomarker is chitotriosidase, a lysosomal hydrolase with the capability to degrade chitin, a part of the cell wall of lower organisms. Its levels are 100- to more than 4000-fold increased in symptomatic Gaucher disease patients, with minimal elevations in patients with asymptomatic disease. In situ hybridization and histochemistry of bone marrow aspirates and sections of spleen from Gaucher disease patients revealed that chitotriosidase is very specifically produced by storage cells\textsuperscript{24}. This is also supported by the close linear relationship between levels of chitotriosidase and glucosylceramide in different sections of spleen from Gaucher disease patients. The plasma chitotriosidase level does not reflect one particular clinical symptom of Gaucher disease, suggesting that it rather reflects the sum of the enzyme secreted by Gaucher cells in various body locations\textsuperscript{24}. The problem of apparent substrate inhibition, a phenomenon that prohibited accurate quantification of chitotriosidase levels using conventional substrates, has been solved by the use of a novel substrate, 4-methylumbelliferyl-(4-deoxy)chitobiose\textsuperscript{25}. Currently, the international recommendations for the follow-up of patients include chitotriosidase as the most valuable biomarker\textsuperscript{26}. In this thesis, a dose response effect for chitotriosidase was established\textsuperscript{12}. A shortcoming of this marker is the fact that 6\% of the population is deficient for chitotriosidase, lacking any activity\textsuperscript{27}. Carriers of this mutation will display lower chitotriosidase activities, roughly being 50\% of wildtype patients\textsuperscript{28} and in situations where absolute values are used in clinical decision making, these issues should be carefully considered. In patients that are completely deficient, monitoring of plasma CCL18 now seems a good and reliable alternative that can aid in the clinical management of Gaucher disease patients\textsuperscript{29}. The almost perfect linear relationship with relative chitotriosidase levels and the finding that CCL18 is indeed produced by Gaucher cells make it a good substitute for the assessment of Gaucher cell burden. Deegan and co-workers even claimed that CCL18 is superior to chitotriosidase\textsuperscript{30}. They stated that, in addition to the fact that CCL18 was detectable in all patients, the reductions in CCL18 more closely reflected the reductions in visceral organs and platelet counts. However, for excess liver volume chitotriosidase was superior, while the relationship of platelet response with chitotriosidase was not given. Since the bone marrow response was not taken into account, we feel that chitotriosidase levels still provide the best indicator of overall Gaucher cell burden and the most sensitive tool for follow up. In the case of chitotriosidase deficiency, CCL18 is the second best biomarker.
Another novel possible marker for Gaucher cell burden is the macrophage/monocyte specific sCD163. sCD163 was also shown to be strongly elevated in Gaucher disease patients, with decreases after start of enzyme replacement therapy. There is a strong correlation with chitotriosidase. Apart from the number of Gaucher cells, sCD163 levels are probably also influenced by the high degree of proteinase activity in Gaucher serum that contribute to the shedding of sCD163 from the surface of monocytes/macrophages, thereby leading to increased plasma levels.

Clearly, markers for total Gaucher cell burden have become readily available, although their predictive value warrants more research. Disease compartment specific markers however, are absent. This is relevant since changes in disease severity are usually not reflected in all parameters at the same time or to the same extent. Especially the evaluation of bone disease, necessitating highly advanced imaging techniques, would be greatly facilitated by a bone specific marker. Cathepsin K, involved in osteolysis, and tartrate resistant acid phosphatase (TRAP), which is known to be secreted by osteoclasts, have been implicated in the pathophysiology of Gaucher bone disease. However, a link with severity of skeletal disease has not been established. In that respect, the chemokines MIP-1α and MIP-1β are most promising. The fact that MIP-1β can differentiate prior to start of enzyme replacement therapy between patients with an adequate bone response versus those with ongoing severe bone disease, strongly suggest that these are not merely the products of past bone damage. Interestingly, MIP-1β, in contrast to chitotriosidase and CCL18, is not produced by Gaucher cells but rather by classically activated macrophages surrounding the mature storage cells. Our observation that plasma MIP-1β levels improved faster during the first year of treatment in patients using high-dosed ERT versus those on a low dose, indicated that not only the initial correction of Gaucher cells but also that of associated phagocytes is enzyme dose-dependent.

In practice, biomarkers should not be the sole factor on which clinical decisions are made. Information concerning the change in levels of markers should always be combined to clinical data concerning skeletal disease, hematology and organomegaly. On the other hand, biomarkers may provide useful information on residual disease status that may not always be reflected by more readily appreciated clinical data and as such should guide the clinician in their search for compartment related storage.

Section III. Associated morbidities
Now that Gaucher disease can be effectively treated in the majority of patients, the focus has been shifted from the main clinical features, hepatosplenomegaly, cytopenia and bone disease, to the morbidity and mortality that is caused by associated diseases. This has become even more important with the recent finding that, in contrast to what was generally accepted, life expectancy in Gaucher disease patients is shortened (69.7 years,
It is already known from earlier studies \cite{36,37} that Gaucher disease patients carry an abnormal lipid profile with low HDL cholesterol levels and, to a lesser extent, low LDL cholesterol levels. In addition, the major apolipoproteins related to these cholesterol particles, ApoA1 and ApoB are decreased. ApoE levels, however, are elevated \cite{36}. It has been hypothesized, but never demonstrated, that the increased number of macrophages secrete high amounts of this apolipoprotein, thus facilitating reverse cholesterol transport to the liver by ApoE containing particles \cite{37}. Whether this unfavorable cholesterol profile would lead to enhanced cardiovascular risk has been debated. In this thesis, we described that both risk analysis of cardiovascular events using indirect standardization as well as measurement of carotid-intima media thickness (c-IMT) in a large cohort of Gaucher disease patients, did not result in an increased risk for cardiovascular disease. This is in line with earlier observations showing no increase in coronary heart disease in patients with Gaucher disease \cite{38}. Possibly, the aforementioned increase in reverse cholesterol transport, as well as the anticoagulant state in most Gaucher patients \cite{39} may protect the patients from early atherosclerosis.

The most extensive studied associated condition is cancer. The first large study in 1982 by Lee, described 239 type I Gaucher disease patients from the Gaucher disease registry of whom 32 (13%) had cancer. Haematological malignancies were found in 34% of the cancer patients \cite{40}. In the subsequent years, the study described in this thesis \cite{41}, two studies from Israel \cite{42,43}, a study from the Gaucher registry \cite{44}, and a study from a large US veterans cohort \cite{45} followed. Several factors can attribute to the fact that not all studies found an elevated risk of cancer, or in particular of haematological malignancies. The study from the Gaucher registry \cite{44} probably underestimated cancer incidence since data entry into the registry by doctors is voluntary, and not specific for cancer. In the study in US veterans, an impossibly high number of Gaucher patients was described. This was probably due to the fact that Gaucher patients were identified using ICD discharge codes that also coded for other lysosomal storage disorders as well as lipid disorders such as hypercholesterolemia \cite{45}. Although not proven, Gaucher disease severity could be a risk factor for developing cancer. If so, one would expect lower relative risks from the study in US veterans, as well as from the two Israeli studies \cite{42,43}, where on average, patients have milder disease due to the more commonly found N370S mutation in this population. In addition, since the development of cancer in Gaucher disease is related to age \cite{41}, increased risks will not be found in a relatively young Gaucher disease cohort.

Besides the increased risk of cancer, especially of multiple myeloma, other immunoglobulin abnormalities, such as polyclonal gammopathy and the pre-malignant monoclonal gammopathy of undetermined significance (MGUS), are prevalent in Gaucher disease...
(for a review, see chapter 4 of this thesis). This has led to a theory suggesting that the accumulated lipid is the source of chronic antigenic stimulation of macrophages. The macrophage derived cytokines initiate an immunological cascade leading subsequently to hypergammaglobulinemia, MGUS and finally B-cell malignancy. For other diseases that are characterized by chronic inflammation, the relationship with cancer has been established, with a higher the risk of associated carcinogenesis the longer the inflammation persists.

The hypothesis prompts several questions; is the accumulated lipid indeed the starting point of the derangements? Which mediators are important and by which cells are they produced? Does enzyme replacement therapy have a beneficial effect on these processes? Support for the first question comes from a study showing that murine macrophages that were stimulated in vitro by glucocerebrosidase, release the pro-inflammatory lymphocyte activating factor (LAF = Interleukin (IL)-1) in a dose-responsive manner. This effect was not seen on incubation with galactocerebroside, sphingomyelin and ceramidetrihexoside. Massive or longstanding lipid accumulation is probably not required since 91% of paediatric patients (average age 8.5 years) already had a polyclonal gammopathy. The lack of an association between disease severity or exposure time and the occurrence of MGUS corresponds to these findings (chapter 4). What’s more, a glucocerebrosidase deficient mouse (L444P homozygote) showed evidence of B-cell proliferation, as well as elevated serum IgG levels, even though storage cells were not found.

The exact identification of the mediators and their role in pathophysiology is far from understood. A number of cytokines, chemokines and growth factors have been studied, all of which are involved in either inflammation or multiple myeloma. Due to differences in methods it is difficult to compare results and find the common denominators. Two mediators that have been consequently found to be elevated are interleukin (IL)-10 and CCL18. IL-10, produced by T cells and activated macrophages, is an anti-inflammatory cytokine. It also has pro inflammatory capacities, promoting the activation and differentiation of B cells and the growth of myeloma cells (for review on IL-10, see). Furthermore, it is involved in multiple myeloma bone disease. Possibly, the continuous low grade inflammation in Gaucher disease is counteracted by IL-10, with unwanted side effects on bone homeostasis and B-cell function. CCL18 is chemotactic for lymphocytes, particularly naive T cells and B cells, and for immature dendritic cells, and could thus be involved in initiating the adaptive immune response. It is unlikely that the inflammation is directly caused by Gaucher cells, since Gaucher cells have a phenotype similar to anti-inflammatory alternatively activated macrophages. More likely, the disruption of tissue architecture and function leads to activation of surrounding macrophages. These cells in turn elicit a pro-inflammatory response, with Gaucher cells counteracting the inflammatory compounds.
The development of hepatocellular carcinoma in our patients, in the absence of risk factors such as cirrhosis, chronic hepatitis B or C or irradiation, seems to be related to massive Gaucher infiltration of the liver after splenectomy. Iron overload could also be important for the development of hepatocellular carcinoma. Most Gaucher disease patients show increased plasma ferritin levels. It is presently unclear whether the high ferritin reflects increased storage of iron or merely an aspecific acute phase response. Nevertheless, excess ferritin leads to decreased phagocytosis and altered T cell subsets in Gaucher disease which may affect anti tumor surveillance. In addition, long standing iron deposition is associated with an increased risk of fibrosis, cirrhosis and hepatocellular carcinoma. In our cohort, no differences were found in ferritin levels between patients with or without a monoclonal gammopathy.

The increased risk of malignancies, especially multiple myeloma has recently led to a consensus statement by an international panel of Gaucher experts. Measures that aim for early recognition of haematological malignancies and MGUS were suggested. We would like to add that screening for hepatocellular carcinoma by determination of serum α-fetoprotein should also be performed at regular intervals.

**Suggestions for future studies.**

Within the group of lysosomal storage diseases, Gaucher disease has been a frontrunner in the development of new therapies. Momentarily new enzyme preparations, substrate inhibitors, as well as small molecules that can serve as chaperones are under investigation. Chaperones are believed to bind to the active site of glucocerebrosidase variants stabilizing their three-dimensional structure in the endoplasmic reticulum, thereby preventing their degradation and allowing proper trafficking to the lysosome. Hopefully, the launch of these new therapies will be accompanied by adequate studies comparing the different preparations and dosing regimens. At the very least, data from clinical studies should be collected and made openly accessible for comparison. Ultimately, clinicians should be able to choose the right product or combination of products for the right patient, taking into account disease severity, assessment of expected therapeutic response and convenience.

Other important research questions regarding therapy are whether therapy prevents the occurrence and severity of malignancies and gammopathies. Results from this thesis suggest a there might be such a favourable effect. In a recent study in children, immunoglobulin levels (in particular IgM and IgA) decreased during enzyme replacement therapy. Evidence that these changes are clinically relevant and justify early or high dose treatment cannot be provided by current knowledge. Larger studies, warranting an international multi centre effort, are thus needed fro confirmation. Again, collaborative efforts are needed to address these issues.
Another challenging research area is the adequate treatment of bone disease. The group of non responders concerning bone disease should be analyzed more thoroughly and in a larger cohort. The effect of dose reductions in these patients should be prospectively studied. The best strategy to improve bone disease is obviously to start therapy before severe infiltration of the bone compartment, as evidenced by low fat fraction or BMB, has occurred. It has become clear that bone disease is less reversible as compared to other manifestation of the disease, and as such should be identified as soon as possible. In that respect, it should be stressed once more that there is no straight forward relationship between visceral and skeletal involvement. For that reason, every Gaucher patient should be screened for the existence of early signs of bone involvement. When bone disease is already in an advanced stage, improved targeting of enzyme therapy, newly developed oral preparations or a combination of both should be further explored.

With respect to the evaluation of treatment response, the predictive value of disease markers warrants more study. Especially MIP-1β could prove valuable as a predictor of deterioration of bone disease and should be studied more extensively. For better understanding and use of markers such as chitotriosidase and CCL18, means to accurately assess total Gaucher body burden are necessary. At this moment no golden standard exists and it unclear what the different Gaucher compartments contribute to the number of storage cells. In the past a severity score index (SSI67) has been established, based upon the extent of cytopenia, organomegaly and bone disease. It is a very practical scoring system because it does not require advanced imaging techniques, such as CT or MRI. However, the score has not been validated or closely related to the storage load. The development of a score that more adequately reflects the overall burden of storage cells may be of great value.

It is incompletely understood how the enzymatic defect with subsequent storage of glucocerebroside ultimately leads to the clinical expression of the disease. In the light of this thesis, the development of bone disease as well as the mechanism causing gammopathies and malignancies is of particular interest. Mouse models, including one that was recently developed by postnatal induction of mutations in the glucocerebrosidase gene, resulting in the formation of storage cells in various tissues68, possibly will provide important tools for future functional studies.

Reference List


