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Publication date
2024

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Citation for published version (APA):
Wegdam, W. (2024). *In search of protein biomarkers in ovarian cancer and Gaucher disease*. [Thesis, fully internal, Universiteit van Amsterdam].

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Gaucher Disease: A Model Disorder for Biomarker Discovery

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Expert Review of Proteomics. 2009; Vol 6 (4): 411–19.

Abstract

Gaucher disease is an inherited lysosomal storage disorder, characterized by massive accumulation of glucosylceramide-laden macrophages in the spleen, liver and bone marrow as a consequence of deficient activity of glucocerebrosidase. Gaucher disease has been the playground to develop new therapeutic interventions such as enzyme-replacement therapy and substrate-reduction therapy. The availability of these costly therapies has stimulated research regarding suitable biomarkers to monitor onset and progression of disease, as well as the efficacy of therapeutic intervention. Given the important role of storage cells in the pathology, various attempts have been made to identify proteins in plasma or serum reflecting the body burden of these pathological cells.

In this review, the existing data regarding biomarkers for Gaucher disease, as well as the current application of biomarkers in clinical management of Gaucher patients are discussed. Moreover, the use of several modern proteomic technologies for the identification of Gaucher biomarkers is reviewed.

Introduction

In man, at least 40 distinct inherited diseases occur that are caused by impaired lysosomal catabolism, so-called lysosomal storage disorders [1]. The most prevalent subgroup is made up of sphingolipidoses, inherited disorders characterized by excessive accumulation of one or multiple (glyco)sphingolipids. Particularly prominent is Gaucher disease [2]. After the first clinical case description by Philippe Gaucher in 1882, it was soon realized this was an example of a distinct disease entity, subsequently designated Gaucher disease [2]. The primary storage material in Gaucher disease was identified as glucocerebroside (glucosylceramide) in 1934. This glycosphingolipid is the common intermediate in the synthesis and degradation of gangliosides and globosides [2]. In 1965, Patrick and Brady and colleagues independently showed that the primary defect in Gaucher disease is a marked deficiency in activity of the lysosomal enzyme glucocerebrosidase (EC 3.2.1.45) [3,4]. The Gaucher disease diagnosis can be confirmed by the demonstration of deficient glucocerebrosidase activity towards an artificial, fluorogenic substrate, 4-methyl-umbelliferyl-β-d-glucoside in cells, tissues or urine samples [5,6].

Inherited deficiencies in glucocerebrosidase result in the accumulation of its lipid substrate in the lysosomal compartment of macrophages throughout the body. Different phenotypes (types I, II and III) are generally recognized, which are differentiated on the basis of the presence or absence of neurological symptoms. More recently, it has been realized that a complete deficiency in glucocerebrosidase activity also occurs, resulting in major skin permeability abnormalities with lethal consequences either prenatally or shortly after birth [7]. The prevalent Gaucher phenotype is the non-neuronopathic type I Gaucher disease. Age of onset and severity of clinical manifestations are highly variable. Characteristic symptoms include splenomegaly with anemia and thrombocytopenia, hepatomegaly and bone disease. Anemia may con- tribute to chronic fatigue. Thrombocytopenia and prolonged clotting times can lead to an increased bleeding tendency. Atypical bone pain, pathological fractures, avascular necrosis and extremely painful bone crises may also have a great impact on the quality of life [2].

The *GBA1* gene encoding glucocerebrosidase is located at the chromosomal locus 1q21. Numerous mutations in the *GBA1* gene have been identified in relation to Gaucher disease. It has become clear that the underlying mutations in the *GBA1* gene partly correlate with the severity of disease manifestation and,

in particular, development of neurological symptoms. A low residual enzyme activity in leukocytes or cultured fibroblasts is associated with a more severe disease course [8,9]. In contrast to other lysosomal glycosidases, GBA1 does not acquire mannose-6-phosphate moieties, but is sorted and trans- ported to lysosomes by interaction with the integral membrane protein LIMP-2 [10–12]. LIMP-2 deficiency may, therefore, also result in reduced cellular GBA1 activity [13]. Since GBA1 requires the activator protein saposin C for efficient intralysosomal degradation of glucosylceramide, deficiency in this accessory protein also results in glucosylceramide accumulation in cells [14]. The majority of Gaucher patients has one N370S *GBA1* allele and develops a non-neuropathic, type I disease. In these patients, accumulation of the substrate glucosylceramide is restricted to tissue macrophages.

Type I Gaucher disease is relatively common in all ethnic groups. It is prevalent among Ashkenazi Jews, with a carrier frequency as high as one in 15 and an incidence of approximately one in 1000. The most common mutation in the *GBA1* gene of Caucasians, including Ashkenazi Jews, encodes the amino acid substitution N370S [15]. Heteroallelic presence of the N370S mutation is always associated with a non-neuronopathic course [9,15]. Most, but not all, homozygotes for the N370S mutation develop significant clinical symptoms. Twin studies and the poor predictive power of phenotype–genotype investigations in Gaucher disease clearly showed that epigenetic factors also play an important role in Gaucher disease manifestation [16–18].

Although glucocerebrosidase is present in lysosomes of all cell types, type I Gaucher disease patients develop storage of glucosylceramide in macrophages only. It is believed that the storage material stems from breakdown of exogenous lipids derived from the turnover of blood cells. Recently, the molecular entity of the ubiquitous nonlysosomal glucocerebrosidase (GBA2) has been identified [19–21]. This enzyme most likely protects most cell types of Gaucher patients, with the exception of macrophages, from massive glucosylceramide accumulation. The glucosylceramide-loaded macrophages of Gaucher patients show a characteristic morphology with a ‘wrinkled tissue paper’ appearance of their cytoplasm, containing lysosomal inclusion bodies; these cells are referred to as Gaucher cells (Figure 1A) [2]. In recent decades, it has become apparent that Gaucher cells are not inert containers of storage material but viable, chronically activated macrophages that contribute to the diverse clinical manifestations of Gaucher disease. In tissue lesions of Gaucher patients, mature storage cells (i.e., alternatively activated macrophages) are surrounded by newly formed,

highly inflammatory macrophages [22,23]. Consistent with these observations, Gaucher patients show increased plasma levels of several proinflammatory and anti-inflammatory cytokines, chemokines and hydrolases [23–31]. Factors released by Gaucher cells and the surrounding macrophages are thought to play crucial roles in the development of such common clinical abnormalities in Gaucher patients as osteopenia, activation of coagulation, hypermetabolism, gammopathies, multiple myeloma and hypolipoproteinemias [27].

Therapies of type I Gaucher disease have been developed that aim to correct Gaucher cells or at least prevent further formation of storage cells. Nowadays, type I Gaucher disease is successfully treated by enzyme-replacement therapy (ERT) and substrate-reduction therapy. ERT is based on chronic intravenous administration of macrophage-targeted recombinant glucocerebrosidase (Cerezyme®; Genzyme Corp.) [32]. Substrate-reduction therapy is based on chronic oral administration of *N*-butyl-deoxynojirimycin (Zavesca®; Actelion), which inhibits glycosphingolipid biosynthesis [33,34]. ERT is still considered the best choice of treatment in more severely affected patients [35]. The impressive clinical responses following ERT and substrate-reduction therapy substantiate the concept that Gaucher cells underlie disease manifestation and progression in Gaucher patients.

Considerable attention is currently focused on an alternative therapeutic approach, the so-called chaperone therapy. This therapy is based on the concept that an active site-directed inhibitor of an enzyme (a pharmacological chaperone) can already bind an enzyme during folding in the endoplasmic reticulum and, thus, stabilize the proper protein conformation. This stabilization of the critical region of the enzyme during folding might result in a larger percentage of properly folded enzymes reaching the lysosome. Several of the Gaucher-associated mutations in the *GBA1* gene result in impaired folding of GBA1 in the endoplasmic reticulum. Chemical chaperones have been demonstrated to work for several common mutated forms of GBA1, albeit only in artificial cellular assays [36,37].

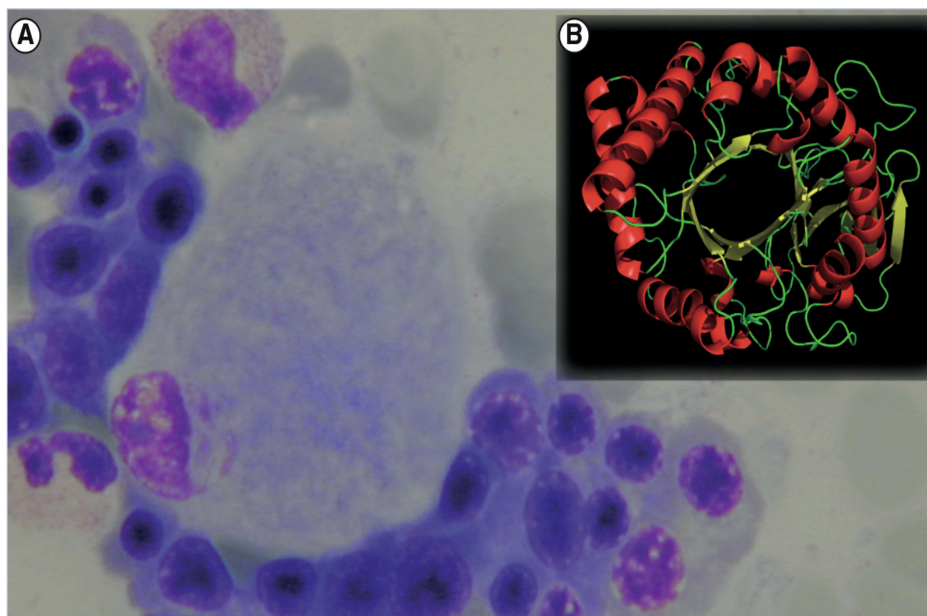

Figure 1. (A) Typical Gaucher cell from a bone marrow aspirate with characteristic morphology and 'wrinkled tissue paper' appearance of its cytoplasm. **(B)** Chitotriosidase core domain, a (β/α)8 (TIM)-barrel enzyme secreted by Gaucher cells that was found to play a role in innate immunity [38–40].

Biomarkers of Gaucher cells

Given the prominent role of Gaucher cells in the pathophysiology of the disorder, considerable attention has been focused on the identification of plasma markers for such macrophages. Abnormalities in levels of tartrate-resistant acid phosphatase, angiotensin-converting enzyme, hexosaminidase, ferritin, ApoE and lysozyme in serum or plasma samples of Gaucher patients had been documented for some time [27,41]. Later on, increased plasma levels of various cathepsins, among which cathepsin D, K and S, were reported for Gaucher patients [30]. All these proteins are known to be produced by macrophages. However, none of them appears to be a truly specific marker for pathological Gaucher cells and their levels in serum of symptomatic Gaucher patients show overlap with those observed in healthy subjects. Their use as biomarkers for Gaucher cells therefore seems restricted.

Chitotriosidase

The need for a very sensitive and specific plasma biomarker for Gaucher cells prompted a further search for such a parameter. This led us to the discovery of

a very marked abnormality in serum of symptomatic Gaucher patients. Serum from such individuals showed a 1000-fold increased capacity to degrade the fluorogenic substrate 4-methylumbelliferyl-chitotrioside [42]. The corresponding enzyme had been hitherto not described and was named chitotriosidase. The chitotriosidase protein was subsequently purified and its cDNA was cloned [43,44]. Chitotriosidase was found to be the human analogue of chitinases from lower organisms. *In situ* hybridization and histochemistry of bone marrow aspirates and sections of spleens from Gaucher patients revealed that chitotriosidase is very specifically produced by storage cells (Figure 1B). This is also supported by the close linear relationship between levels of chitotriosidase and glucosylceramide in different sections of spleens from Gaucher patients [41]. Since tissue glucosylceramide is the best possible quantitative measure for storage cells, it may be deduced that total chitotriosidase production is directly proportional to the amount of Gaucher cells. In a culture model of Gaucher cells chitotriosidase amounts for almost 10% of the total of secreted protein [45]. In sharp contrast, common tissue macrophages and dendritic cells do not produce chitotriosidase. These observations help to understand the very specific, gross elevation in chitotriosidase activity in the blood of Gaucher patients. A relation between the total body burden of storage cells in Gaucher patients and their plasma chitotriosidase levels has been noted. The plasma chitotriosidase level does not reflect any particular clinical symptom of Gaucher disease, suggesting that it rather reflects the sum of secreted enzyme by Gaucher cells in various body locations [42].

Plasma chitotriosidase can be determined by monitoring the hydrolysis of chito-oligosaccharides, or more conveniently that of the fluorogenic substrate 4-methylumbelliferyl-chitotrioside [46]. However, the ability of chitotriosidase to transglycosylate as well as hydrolyze this substrate complicates the enzyme assay [47]. Special care has to be taken to ensure that the enzyme activity is truly proportional to the amount of chitotriosidase protein. A far more convenient, sensitive and accurate detection is feasible by measuring the activity of chitotriosidase towards the recently designed fluorogenic substrate 4-methylumbelliferyl- deoxy-chitobioside [47]. Interpretation of plasma chitotriosidase levels is intrinsically complicated by the common occurrence of a particular 24-bp duplication in the chitotriosidase gene, preventing the formation of chitotriosidase protein [48]. In most ethnic groups, approximately one in every three individuals carries this abnormality and approximately one in every 20 individuals, including Gaucher patients, is homozygous for this trait [48]. It has been established that carriers of the 24-bp duplication have half the

amount of plasma chitotriosidase activity detected in individuals with a wild-type chitotriosidase genotype [49]. It is, therefore, common to correct plasma chitotriosidase activity by multiplying it by two in the case of Gaucher patients who are carriers of the 24-bp duplication [50]. Besides the common 24-bp duplication leading to complete chitotriosidase deficiency, other polymorphisms have been detected of which the G102S substitution is the most frequent (allele frequency: ~ 0.25). This polymorphism leads to an enzyme with a slightly impaired catalytic activity towards the 4-methylumbelliferyl-chitotrioside substrate as compared with wild-type [51,52]. However, the activity of the G102S enzyme was normal when using 4-methylumbelliferyl-deoxy-chitobioside as the substrate. It should be mentioned that increased plasma chitotriosidase activity is not unique for Gaucher patients. Plasma chitotriosidase activity is increased, albeit much more modestly, in several lysosomal [53–59] and nonlysosomal diseases such as sarcoidosis, visceral Leishmaniasis, leprosy, arthritis, multiple sclerosis, thalassemia, COPD, malaria and atherosclerosis [42,60–69].

PARC/CCL18

A subsequent search using SELDI-TOF mass spectrometric analyses led us to the discovery of massive overproduction and secretion by Gaucher cells of the chemokine PARC/CCL18, of which the mRNA was previously observed to be upregulated in the spleen of a patient with Gaucher disease [28,30]. This plasma PARC/CCL18 can not be reliably quantified using SELDI-TOF, but reliable quantification is obtained by ELISA [70]. Plasma PARC/CCL18 levels are ten- to 40-fold elevated in symptomatic Gaucher patients [28,71]. Due to its basic nature and small molecular mass, PARC/CCL18 levels in urine are proportional to those in the circulation. Therefore, urinary PARC/CCL18 excretion offers insight into the body burden of Gaucher cells [72]. Measurement of plasma PARC/CCL18 has been found to yield an excellent additional tool to monitor changes in body burden of Gaucher cells. It is particularly useful for the evaluation of those patients who are chitotriosidase deficient [71].

MIP-1α & MIP-1β

Very recently, van Breemen and coworkers reported markedly elevated levels of the chemokines MIP-1α and MIP-1β in plasma of symptomatic Gaucher patients [23]. Interestingly, with immuno- histochemistry these proteins were found to be produced by sur- rounding inflammatory spleen macrophages and not by mature Gaucher cells [23]. The different cellular source is also reflected in the observation that plasma chitotriosidase and PARC/CCL18, both stemming from Gaucher cells, respond comparably to ERT. However, corrections in plasma

MIP-1α and MIP-1β following ERT are not proportional to those found with the true Gaucher cell biomarkers [23]. Fascinatingly, a relation was observed between plasma MIP-1β and skeletal disease: stable high plasma MIP-1β levels in spite of prolonged ERT were found to correlate with ongoing skeletal disease [23]. Clearly, rigorous analysis of a large cohort of Gaucher patients is required to establish the value of plasma MIP-1β as a biomarker, especially its value as a prognostic marker for skeletal response to therapy.

Application of present biomarkers in clinical management

To date, biomarkers are already widely used in the clinical management of several conditions. The most obvious example is the measurement of blood glucose and/or glycated hemoglobin in diabetic individuals. These assessments guide clinicians in decision-making regarding initiation and optimization of therapeutic interventions. A less obvious example is found in MRI techniques. It should be realized that MRI is based on nuclear magnetic resonance of molecules and should be viewed as a chemical assessment. One striking example of this is quantitative chemical shift imaging that allows assessment of local fat concentration [73]. Although in daily practice biomarkers are widely imaged and assessed in the modern clinic, there remains a lively debate among advocates and opponents of the use of imaging and assessments of chemical structures to support clinical care. It is evident that proposed biomarkers should not be too hastily adopted in clinical decision-making and that sound proof of their true value has yet to exist. Rigorous validation of the relationship between a proposed biomarker and disease activity and outcome is of key importance. However, it must be emphasized that biomarkers should assist in, and not strictly direct, clinical management.

Plasma chitotriosidase measurement is nowadays commonly employed as a first screen in the diagnosis of Gaucher disease. Increasing plasma levels seem to reflect gradual accumulation of storage cells in the patient's body. In an attempt to assess the utility of plasma chitotriosidase activity measurement as a biomarker for treatment efficacy, Hollak and coworkers investigated the relationship between enzyme activity and clinical parameters [74]. In patients with high clinical severity scores, chitotriosidase levels were usually above 20,000 nmol/ml/h and always above 15,000 nmol/ml/h, whereas patients with less severe disease tended to have lower values. During enzyme- supplementation therapy, the mean decrease in 12 months was 32% (range: 0–82%) and 78% of patients had a decrease of more than 15%. In six patients with a decrease in chitotriosidase activity of less than 15%, the clinical response to treatment was

inferior to that of other patients, with less reduction in organomegaly in four patients and bone problems in two patients. In addition, the chitotriosidase response was related to the severity of the disease; less reduction in plasma activity was seen in more severely affected individuals. On the basis of this investigation, it has been proposed that in patients in whom the initiation of treatment is questionable based solely on clinical parameters, a chitotriosidase activity above 15,000 nmol/ml/h may serve as an indicator of a high Gaucher cell burden and an indication for the initiation of treatment [74]. A reduction in chitotriosidase activity of less than 15% after 12 months of treatment, in combination with an insufficient response of at least one clinical parameter, should be a reason to consider dose increase. Furthermore, a sustained increase in chitotriosidase at any point during treatment should alert the physician to the possibility of clinical deterioration and the need for dose adjustment. A more recent retrospective analysis by Deegan and coworkers confirmed the value of the use of plasma chitotriosidase in Gaucher disease management and presented evidence for a comparable use of PARC/CCL18 [71].

A recent report by two treatment centers, the Academic Medical Center (Amsterdam, The Netherlands) and the Heinrich-Heine University (Düsseldorf, Germany) on the long-term outcome of different ERT dosing regimens revived discussion on biomarkers for Gaucher disease [49,75,76]. The study revealed that improvement in hemoglobin, platelet count and hepatosplenomegaly was not significantly different between both cohorts, whereas plasma chitotriosidase and bone marrow involvement by MRI improved more quickly and was more pronounced in the higher dosed group [49]. Given the concerns regarding very high costs associated with ERT and given the acceptable clinical outcome of low dose ERT, Zimran and colleagues argued that surrogate markers of disease like chitotriosidase are of little value [75]. In their view, there is no clinical necessity for the more rapid and pronounced removal of Gaucher cells accomplished by a higher ERT dosing regimen [77,78]. Indeed, in the case of type I Gaucher disease most clinical manifestations, except some skeletal complications, are reversible. One may therefore question whether there is any need for rapid Gaucher cell reduction. It should be kept in mind that plasma markers of storage cells such as chitotriosidase and PARC/CCL18 offer clinicians insight in the body burden of Gaucher cells and may assist clinicians in the vital decision-making on initiation of treatment as well as optimization of therapy for the individual patient. This could be of particular use in splenectomized patients, since these patients typically have normal platelet counts and hemoglobin levels.

Metabolite biomarkers of Gaucher disease

Increased plasma concentrations of glucosylceramide, the primary storage lipid, have been documented for Gaucher patients [79]. Plasma glucosylceramide is not used as a biomarker since its increase generally is not very pronounced. Moreover, the exact relation between circulating glucosylceramide and storage cells in tissues is far from clear. Interestingly, besides glucosylceramide the ganglioside GM3 is also elevated in Gaucher plasma samples [80]. Increases of GM3 have also been noted in spleens of Gaucher patients. This secondary elevation of GM3 may not be without consequences. Elevated glycosphingolipids such as GM3 are thought to cause insulin resistance [81]. Indeed, a recent study revealed that Gaucher patients are insulin resistant without overt hyperglycemia [82].

Discovery of additional protein biomarkers for Gaucher disease

Ongoing attention is paid to the detection of useful protein biomarkers for Gaucher disease. We and others have used ana- lysis of gene expression in storage cells [30]. Another approach is a thorough survey of protein composition of bodily fluids, or cell and tissue specimens of symptomatic Gaucher patients. The latter approach has more recently become feasible by the availability of mass spectrometric techniques that allow accurate analysis of proteins, even in complex mixtures like plasma and urine samples. The challenge using plasma samples is particularly daunting. It has been approximately calculated that over 106 different protein molecules reside in plasma and that the dynamic range (difference between the highest and lowest concentration) is at least 1010 [83]. Approximately half of the total protein mass in plasma is accounted for by one protein (albumin, present at∼55,000,000,000 pg/ml), while about ten proteins together make up 90% of the total. At the other extreme of the concentration histogram are the cytokines, such as IL-6, which is normally present at 1–5 pg/ml.

During the course of our investigations, we made use of a variety of proteomic methods to analyze plasma and spleen samples obtained from Gaucher patients. First, 2D gel electrophoresis followed by identification of proteins of interest using peptide mass finger printing led to identification of elevated levels of several cathepsins in spleens from Gaucher patients [Speijer, Unpublished Data] [84]. Analysis of plasma with this technique pointed out that samples from symptomatic Gaucher patients contain a large amount of cathepsins and other proteases. Using the classical procedure with 8 M urea-treated plasma, several high-molecular- weight proteins were absent from Gaucher plasma specimens,

while additional low-molecular-weight proteins became visible. The latter were identified as proteolytic degradation products. By contrast, the presence of 2.2 M thiourea/7.7 M urea in the rehydration solution prevented this phenomenon. Apparently, in the 'urea only' solution, protease(s) uniquely present in Gaucher plasma appear to be still active towards other denatured plasma proteins at low pH [84].

Next we employed SELDI-TOF mass spectrometry (MS) for the analysis of Gaucher plasma samples. In a fast and economic manner a subproteome of plasma can be obtained by SELDI- TOF MS. This typically results in data sets with very low samples-to-variables ratio. To avoid erroneous conclusions due to the undersampling, thorough statistical validation of discrimination models is crucial, as was illustrated recently [85]. A dataset containing serum samples from Gaucher patients and healthy controls served as a test case. Double cross-validation showed that the sensitivity of the model is 89% and the specificity is 90%. Permutation and double cross-validation proved to be crucial to avoid erroneous conclusions stemming from the undersampling. Interestingly, although the study revealed the presence of a very distinctive plasma subproteome in Gaucher patients it rendered no good candidates for specific biomarkers. Upon analysis of the top ten proteins contributing to the discrimination between the normal and Gaucher plasma subproteomes, it was found that they were all relatively small (molecular masses below 10,000 Da) and upregulated in Gaucher patients. As discussed earlier, it is known that various proteases, particularly cathepsins, are elevated in Gaucher plasma [30,84]. This may conceivably lead to unique low- molecular-mass degradation products.

More recently, label-free liquid chromatography (LC)-MS quantification methods have been developed. These methods are typically based on determining peak-area ratios of the same pep- tides between different conditions. The quantitative reproducibility of these methods depends upon the peptide cluster efficiency, which is determined, on one hand, by the mass measurement accuracy and precision, and by the chromatographic retention time reproducibility obtained during the experiment, on the other hand. It was recently discovered that such a label-free approach allows an accurate estimation of absolute protein concentrations in complex mixtures [86]. Using a label-free LC-MS approach (so-called LCMSE), a series of plasma specimens from type I Gaucher patients prior and after therapy were studied [87]. Marked therapy-induced differences were noted in the Gaucher disease protein plasma profile. Comparison with the normal plasma profile revealed that many of the protein abnormalities in

symptomatic patients were at least partially corrected by successful therapy [87]. Importantly, the absolute levels of chitotriosidase protein detected by label-free LCMSE were similar to those expected based on measured enzyme activity in specimens. Proportional therapy- induced changes were noted in 12 proteins from the complement and coagulation cascades [87]. In hindsight, this finding is not surprising since Gaucher patients show a low level of coagulation activation [26]. Grouping of peptides according to their changes in concentrations across conditions (e.g., during therapy) apparently reveals pathways like complement activation. It should be stressed that the dynamic range of presently detectable proteins in plasma with current proteomic techniques, including LCMSE, is still limited. Only the more abundant plasma proteins are quantifiably detected and the challenge for the future is to develop intelligent fractionation approaches that will allow detection of additional less abundant plasma proteins. To emphasize the latter, LCMSE is presently employed to study the proteome of laser capture dissected Gaucher cells from spleens of Gaucher patients. Preliminary analysis of the data reveals that proteins known to be strongly expressed by Gaucher cells are nicely detected and quantified. Moreover, also a number of novel potential protein markers for the storage cells have been identified by the LCMSE. The findings are currently being validated by independent techniques (ELISA, western blots and RT-PCR) and it is investigated whether these proteins can also be found elevated in plasma of symptomatic Gaucher patients.

Expert commentary

Gaucher disease is a very attractive model for the identification of biomarkers, given the gross accumulation of pathological macro- phages in various tissues. Numerous abnormalities in plasma of Gaucher patients occur. The ability of analytic techniques to detect (novel) protein biomarkers can be nicely examined using plasma specimens of symptomatic Gaucher patients. Moreover, the existence of an effective therapy that reduces pathological cell burden allows solid validation of tentative biomarkers. Research on Gaucher disease has indeed already led to the identification of two very specific protein biomarkers of storage cells in plasma of patients. Both chitotriosidase and PARC/CCL18 are found to be produced by the pathological storage cells and their plasma levels correlate with disease manifestation. Measurement of plasma chitotriosidase and PARC/CCL18 levels offers valuable additional tools for clinicians in decision-making during patient management. More recently, additional protein abnormalities in relation to Gaucher disease have been observed. The clinical

value of plasma MIP-1β levels as a marker of ongoing skeletal disease in Gaucher patients awaits further validation by analysis of a larger cohort of patients.

Five-year view

The application of LCMSE proteomics already has led to exciting new findings. It has revealed subtle changes in pathways such as coagulation and has led to the identification of potential novel biomarkers for Gaucher disease. The combination of laser capture dissected Gaucher cells from spleens of Gaucher patients with LCMSE shows that proteins known to be strongly expressed by Gaucher cells are clearly detected and could be readily quantified. In addition, a number of novel potential protein markers for the Gaucher cells have been identified by the LCMSE. These findings warrant further investigations on the consequences of the observed abnormalities. The availability of several different mouse models for Gaucher disease will be of further aid in this. Moreover, novel analytical methods will increasingly identify disease-related abnormalities at the protein level. Only a few of them are expected to prove valuable as genuine biomarkers.

It is conceivable that some detected abnormalities will also render important new insights in pathophysiological mechanisms and in the metabolic adaptations that occur in chronically dis- eased individuals. This expanding information will surely provide novel targets for therapeutic interventions.

Key issues

- Biomarkers are analytes reflecting disease activity.
- In the case of lysosomal lipid storage disorders, biomarkers may be lipid metabolites or proteins secreted by storage cells.
- Biomarkers increasingly fulfill an important role in clinical management.
- Gaucher cells are viable lipid laden macrophages that secrete unique proteins.
- Plasma chitotriosidase activity has been validated as a biomarker for the presence of pathological Gaucher cells.
- Plasma PARC/CCL18 is an attractive alternative for plasma chitotriosidase as a Gaucher cell biomarker.
- Innovative proteomic methods (e.g. LC-MSE) allow discovery of novel protein biomarkers for lysosomal storage disorders.
- Newly identified biomarkers may also render new insights in pathophysiological mechanisms.

Acknowledgements

The authors wish to thank their colleagues in the clinical departments and research laboratories in the Academic Medical Center focusing on lysosomal storage disorders. The continuous support by The Netherlands Gaucher Patient society is gratefully acknowledged.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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**** Excellent overview of Gaucher disease.**

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