Detection of biomarkers for lysosomal storage disorders using novel technologies

van Breemen, M.J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter Thirteen

Biomarkers for lysosomal storage diseases: identification and application as exemplified by chitotriosidase in Gaucher disease

Johannes M.F.G. Aerts\textsuperscript{a,b}, Mariëlle J. van Breemen\textsuperscript{b}, Anton P. Bussink\textsuperscript{b}, Karen J.M. Ghauharali\textsuperscript{b}, Richard R. Sprenger\textsuperscript{a,b}, Rolf G. Boot\textsuperscript{b}, Johanna E.M. Groener\textsuperscript{a}, Carla E.M. Hollak\textsuperscript{c}, Mario Maasd, Suzanne Smir\textsuperscript{c}, Huub C.J. Hoeftloot\textsuperscript{c}, Age K. Smilde\textsuperscript{e}, Johannes P.C. Vissers\textsuperscript{f}, Shreyas de Jong\textsuperscript{g}, Dave Speijer\textsuperscript{a,b}, Chris G de Koster\textsuperscript{a,b}

\textsuperscript{a} Clinical Proteomics Facility, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{b} Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{c} Department of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
\textsuperscript{d} Department of Radiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{e} Biosystems Data Analysis, Swammerdam Institute of Life Sciences, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{f} Waters Corporation, MS Technologies Center, Manchester, United Kingdom
Abstract

A biomarker is an analyte that indicates the presence of a biological process, linked to clinical manifestations and outcome of a particular disease. An ideal biomarker provides indirect but ongoing determinations of disease activity. In the case of lysosomal storage disorders, metabolites or proteins specifically secreted by storage cells are good candidates for biomarkers. Potential clinical applications of biomarkers are found in improved diagnosis, monitoring of disease progression, and assessment of therapeutic correction. These applications are illustrated by reviewing the use of plasma chitotriosidase in clinical management of Gaucher patients, the most common lysosomal storage disorder. The ongoing debate on the value of biomarkers in patient management is addressed. Novel analytical methods have revolutionized the identification and measurement of biomarkers at the protein and metabolite level. Recent developments in biomarker discovery by proteomics are described and the future for biomarkers of lysosomal storage disorders is discussed. Besides direct applications for biomarkers in patient management, biomarker searches are likely to render new insights in pathophysiological mechanisms and metabolic adaptations, and may provide new targets for therapeutic intervention.
Biomarkers are generally defined as chemical entities, ranging from simple metabolites to complex proteins and polynucleotides, which indicate the presence of a biological process linked to the clinical manifestations and outcome of a particular disease. An ideal biomarker provides indirect assessment of disease activity and may assist in clinical management. This concept is far from recent. For example, Herman Boerhaave (1668-1738), one of the founding fathers of modern medicine, already stated in his inaugural speech at the University of Leiden in 1703 that: “Chemistry is indispensable for medical science since it renders a spectrum of objective data and offers the most reliable methods for assessment”. This view is now shared by most clinicians and biomedical researchers as well as the authorities. In a recent report on biomarkers, the European Agency for the Evaluation of Medicinal Products (EMEA) states that: “Biomarkers play an increasingly important role in the development of new drugs. It is expected that they will help to increase the rate of success of new developments and to expedite the development of drugs. Also, biomarkers are key in the shift away from the ‘one size fits all’ to ‘the right drug at the right dose in the right patient’ approach. Hence, biomarkers play an important role for scientists and industry in drug development and for regulators in the approval process” (Report on the EMEA/CHMP Biomarkers Workshop, European Medicines Agency, www.emea.eu.int).

To date biomarkers are already widely used in the clinical management of some conditions. An obvious example is the measurement of blood glucose and/or glycated haemoglobin in diabetic individuals. These assessments guide clinicians in decision making on initiation and optimization of therapeutic interventions. A less obvious example of biomarker assessment concerns magnetic resonance imaging (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 (QCSI) that allows assessment of local fat concentration [1]. Although in the modern clinic in daily practice biomarkers are widely imaged and assessed, there is a longstanding reluctance among part of the medical community to accept the growing role of such markers in patient management. There is an ongoing lively debate among advocates and opponents of the use of imaging and assessments of chemical structures to support clinical care. Unfortunately, this discussion is sometimes clouded by subjective arguments. 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 to exist. Rigorous validation of the relationship between a proposed biomarker and disease activity and outcome is of key importance. Moreover, biomarkers should assist in, and not strictly direct, clinical management.

In this review, the identification and application of biomarkers for inherited lysosomal storage disorders is discussed and their merit is illustrated by plasma chitotriosidase in Gaucher disease.
Lysosomal storage disorders: Gaucher disease

The importance of lysosomal catabolic processes is revealed by the existence of at least 40 distinct inherited diseases, the so-called lysosomal storage disorders [2]. The most prevalent subgroup is the sphingolipidoses, inherited disorders that are characterized by excessive accumulation of one or more (glyco)sphingolipids. Particularly prominent is Gaucher disease [3]. After the first clinical case description by Philippe Gaucher in 1882, it was soon realized this was one example of a distinct disease entity, subsequently designated as Gaucher disease. Only in 1934 was the primary storage material in Gaucher disease identified as glucocerebroside (glucosylceramide). This glycosphingolipid is the common intermediate in the synthesis and degradation of gangliosides and globosides. In 1965 Brady and colleagues, and Patrick showed independently that the primary defect in Gaucher disease is a marked deficiency in activity of the lysosomal enzyme glucocerebrosidase (EC 3.2.1.45) [4,5]. Inherited deficiencies in glucocerebrosidase result in 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 become apparent that a complete deficiency in glucocerebrosidase activity can occur, resulting in major skin permeability abnormalities with lethal consequences either prenatally or shortly after birth [6]. 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 anaemia and thrombocytopenia, hepatomegaly and bone disease. Anaemia may contribute 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. Type I Gaucher disease is relatively common in all ethnic groups. It is prevalent among Ashkenazi Jews, with a carrier frequency as high as about 1 in 15 and an incidence of about 1 in 1000. The most common mutation in the glucocerebrosidase gene of Caucasians, including Ashkenazi Jews, encodes the amino acid substitution N370S. The heteroallelic presence of the N370S mutation is always associated with a non-neuronopathic course [7,8]. Many homozygotes for the N370S mutation develop significant clinical symptoms. Twin studies and the poor predictive power of phenotype–genotype investigations in Gaucher disease have clearly pointed out that epigenetic factors also play a key role in Gaucher disease manifestation [9-11].

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 the breakdown of exogenous lipids derived from the turnover of blood cells. Recently, the protein (GBA2) responsible for the ubiquitous non-lysosomal glucocerebrosidase activity has been identified [12-14]. Most likely this enzyme 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 paper’ appearance of
their cytoplasm, which contains lysosomal inclusion bodies; these cells are referred to as Gaucher cells. In recent decades it has become apparent that Gaucher cells are not inert containers of storage material but viable, chronically activated macrophages which contribute to the diverse clinical manifestations of Gaucher disease. In tissue lesions of Gaucher patients, mature storage cells, which are alternatively activated macrophages, are surrounded by newly formed, highly inflammatory macrophages [15,16]. Consistent with these observations, Gaucher patients show increased plasma levels of several pro-inflammatory and anti-inflammatory cytokines, chemokines, and hydrolases [17-20]. Factors released by Gaucher cells and surrounding macrophages are thought to play a crucial role in the development of common clinical abnormalities in Gaucher patients such as osteopenia, activation of coagulation, hypermetabolism, gammopathies and multiple myeloma and hypolipoproteinaemias.

Therapies of type I Gaucher disease have been developed that aim to correct Gaucher cells or at least prevent further formation of storage cells. Type I Gaucher disease is presently successfully treated by enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). ERT is based on chronic intravenous administration of macrophage-targeted recombinant glucocerebrosidase (Cerezyme; Genzyme Corp., Cambridge, MA, USA) [21]. SRT is based on chronic oral administration of N-butyldexoxynojirimycin (Zavesca; Actelion, Basel, Switzerland) that inhibits glycosphingolipid biosynthesis [22,23]. ERT is still considered the first choice of treatment in more severely affected patients [24]. The impressive clinical responses following ERT and SRT substantiate the concept that Gaucher cells underlie disease manifestation and progression in Gaucher patients.

**Biomarkers of Gaucher cells**

Given the prominent role of Gaucher cells in the pathophysiology of the disorder, considerable attention has been focussed on the identification of plasma markers for such macrophages. Abnormalities in levels of tartrate-resistant acid phosphatase (TRAP), angiotensin-converting enzyme (ACE), hexosaminidase and lysozyme in serum samples from Gaucher patients had been documented for some time (for a review see refs [17,25]). More lately, increased plasma levels of various cathepsins, among which cathepsin K, were reported for Gaucher patients [20]. All these proteins are known to be produced by macrophages. However, none of them appears to be a truly specific marker for the pathological Gaucher cells and their levels in serum of symptomatic Gaucher patients may overlap with those observed in healthy subjects. Their use as biomarkers for Gaucher cells is therefore restricted. The need for a very sensitive and specific plasma biomarker for Gaucher cells prompted a search for such parameter. This led us to the discovery of a very marked abnormality in serum of symptomatic Gaucher patients. Serum from such individuals showed a thousand fold increased capacity to degrade the fluorogenic substrate 4-methylumbelliferyl-chitotrioside [26]. The corresponding enzyme had hitherto not been described and was named chitotriosidase. The chitotriosidase protein was subsequently purified and its cDNA was cloned [27,28]. 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 specifically produced by storage cells. This is also supported by the close linear relationship between chitotriosidase and glucosylceramide levels in different sections of spleens from Gaucher patients [25]. Since glucosylceramide is the best possible quantitative measure for storage cells, it may be deduced that chitotriosidase production is directly proportional to Gaucher cell mass. In a culture model of Gaucher cells chitotriosidase amounts for almost 10% of the total of secreted protein. In sharp contrast, common tissue macrophages do not produce chitotriosidase. These observations help to understand the very specific, gross elevation of chitotriosidase 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 one particular clinical symptom of Gaucher disease, suggesting that it rather reflects the sum of secreted enzyme by Gaucher cells in various body locations [26].

Plasma chitotriosidase can be determined by monitoring the hydrolysis of the fluorogenic substrate 4-methylumbelliferyl-chitobioside. However, the ability of chitotriosidase to transglycosylate as well as hydrolyze this substrate complicates the enzyme assay [29]. 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-chitotrioside [29]. Interpretation of plasma chitotriosidase levels is intrinsically complicated by the common occurrence of particular 24-bp duplication in the chitotriosidase gene, preventing the formation of chitotriosidase protein [30]. In most ethnic groups about 1 in every three individuals carries this abnormality and about 1 in every twenty individuals, including Gaucher patients, is homozygous for this trait [30]. It has been established that carriers of the 24-bp duplication show half the amount of plasma chitotriosidase detected in individuals with the wild-type chitotriosidase genotype [31]. It is therefore common to correct plasma chitotriosidase by a factor of two in the case of Gaucher patients which are carriers of the 24-bp duplication [32]. More recently, we discovered the massive overproduction and secretion by Gaucher cells of the chemokine CCL18 [18,33]. Plasma CCL18 levels are 10 to 50-fold elevated in symptomatic Gaucher patients. Measurement of plasma CCL18 has been found to yield an excellent additional tool to monitor changes in body burden on Gaucher cells. It is particularly useful for the evaluation of those patients which are chitotriosidase deficient [34].

Plasma chitotriosidase measurement is nowadays commonly employed as a first screen in the diagnosis of Gaucher disease. Increasing plasma levels 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 [35]. In patients with high clinical severity scores, chitotriosidase levels were usually above 20000 nmol/mL/h and always above 15000 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 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 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, it has been proposed that in patients in whom the initiation of treatment is questionable based solely on clinical parameters, a chitotriosidase activity above 15000 nmol/mL/h may serve as an indicator of a high Gaucher cell burden and an indication for the initiation of treatment [35]. 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 a 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 application of CCL18 [34]. Very recently, van Breemen and coworkers reported markedly elevated levels of the chemokines MIP-1α and MIP-1β in plasma of symptomatic Gaucher patients [16]. Interestingly, these proteins were found to be not produced by mature Gaucher cells, but by surrounding inflammatory cells. A correlation was observed between plasma MIP-1β and extent of skeletal disease. A lack of response in plasma MIP-1β upon enzyme replacement therapy was found to correlate with ongoing skeletal disease [16]. Clearly, rigorous analysis of a large cohort of Gaucher patients is required to establish the value of plasma MIP-1β as biomarker, especially its value as prognostic marker for skeletal response to therapy.

A recent report by two treatment centres, the Academic Medical Center, Amsterdam, The Netherlands and the Heinrich-Heine University, Duesseldorf, Germany, on the long-term outcome of different ERT dosing regimens excited a discussion on biomarkers [32,36,37]. The study revealed that improvement in haemoglobin levels, platelet count, and hepatosplenomegaly was not significantly different between both cohorts, whereas plasma chitotriosidase and bone marrow involvement by magnetic resonance imaging improved more quickly and was more pronounced in the higher-dosed group [32]. Given their 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 [36]. This reasoning is peculiar. Plasma chitotriosidase is without doubt a valid marker of Gaucher storage cells. The comment by Zimran and colleagues should therefore better have been restricted to their belief that there is no clinical necessity for the more rapid and pronounced removal of Gaucher cells that is accomplished using a higher ERT dosing regimen. The discussion on this topic is of broader interest. Fortunately, in the case of type I Gaucher disease most clinical manifestations, except skeletal complications, are thought to be reversible. One may
therefore indeed question whether there is any need for a fast reduction of Gaucher cell by high-dose ERT. However, it seems that in most other lysosomal storage disorders so far, clinical symptoms are not easily reversed by enzyme therapy. Timely intervention seems crucial in these cases, for example in Fabry disease [38]. Plasma markers of storage cells may assist clinicians in the vital decision making on initiation of treatment as well as optimization of therapy for the individual patient. Awaiting irreversible clinical manifestations may not be a good strategy.

Biomarkers and lysosomal storage disorders

Presently considerable effort is spend in the detection of useful biomarkers for other lysosomal storage disorders. Metabolites or proteins specifically secreted by storage cells are good candidates for biomarkers. Use is made of analysis of gene expression in storage cells and/or a thorough survey of protein composition of bodily fluids of symptomatic patients. The latter approach has become more feasible by the recent availability of mass spectrometric techniques that allow accurate analysis of metabolites or proteins in complex mixtures like plasma and urine samples.

Two different classes of potential plasma biomarkers in lysosomal storage disorders should be distinguished. The first category is formed by the primary (or secondary) metabolites that accumulate as consequence of the defect in a particular disease. Good examples are the detection in plasma (or urine) of glucosylceramide, ceramidetrihexoside, glucose tetrasaccharide, and glycosaminoglycans, respectively, in Gaucher disease, Fabry disease, Pompe disease and Mucopolysaccharidoses [39-45]. A common disadvantage of the metabolite marker is that its elevation is not very pronounced, and that the range of normal levels is relatively broad. Moreover, the exact relation between circulating metabolite and storage cells in tissues is far from clear in most cases. In type I Gaucher patients, plasma glucosylceramide correlates with disease severity and chitotriosidase, the protein biomarker of Gaucher cells (JE Groener, unpublished results). In the case of Fabry disease however, plasma ceramidetrihexoside has been found to poorly reflect disease manifestation and therapeutic outcome [46-48].

The second category of potential biomarkers concerns plasma (or urinary) proteins. In the past conventional biochemical methods led to the identification of various protein abnormalities in plasma of Gaucher patients, among them chitotriosidase [17]. The introduction of proteomics, a platform of mass spectrometric (MS) methods suitable for the detection and identification of proteins, has opened new research avenues. Several proteomics techniques for studying plasma proteins have been developed in recent years. The challenge in this respect is daunting. It has been roughly calculated that >10^6 different protein molecules reside in plasma and that the dynamic range (difference between the highest and lowest concentration) is at least >10^10 [49]. Approximately half of the total protein mass in plasma is accounted for by one protein (albumin, present at 55 mg/mL), while about 10 proteins together make up 90% of the total. At the other end of the concentration interval are the cytokines, such as interleukin-6 (IL-6), which is normally present at 1-5 pg/mL. The inherent limitations of present proteomic technologies in
analyzing plasma in depth have recently been nicely reviewed [49]. The oldest, and most robust, approach is fractionation of proteins and mass spectrometric identification of proteins of interest. Fractionation can be accomplished by (multi-dimensional) chromatography or two-dimensional gel electrophoresis. Proteins can be visualized by direct staining in gels or by covalent labelling with fluorescent tags. Protein identification is accomplished by peptide mass finger printing of a tryptic digest or by direct sequencing using nanoHPLC–tandem MS. A number of approaches deserve special attention here since they have been recently employed in a search for plasma biomarkers in a lysosomal storage disorder. Moore and coworkers investigated plasma of children with Fabry disease prior and after ERT using tryptic digestion of plasma protein and differentially labelling peptides with stable isotopes, such that consistent mass differences are introduced into selected amino acid residues [50]. Peptides from samples pre- and post-therapy were then combined to reduce the risk of differences resulting from differential losses during subsequent separation and analysis. The mass differences introduced by the isotope tags could be readily resolved by mass spectrometry and the information used to identify the proteins of origin and define their relative abundance in the samples. Conversion of lysine residues to homoarginine after reaction with O-methylisourea was used as an economical and efficient means of introducing isotope labels into peptides. Except for decreases in α-2-HS glycoprotein, vitamin D-binding protein, transferrin, Ig-α-2 C chain, and α-2-antiplasmin, no other therapy-induced changes in plasma were noted [50]. This elegant study is a nice example of liquid chromatography-mass spectrometry (LC-MS/MS) based relative quantification. The majority of these relative quantification techniques employ the introduction of stable isotopes into the samples such as isotope-coded affinity tags (ICAT), amine-reactive isobaric tagging reagents (iTRAQ), in vivo stable isotope labeling with amino acids in cell culture (SILAC), and ¹⁸O labeling. Recent articles have reviewed stable isotope labeling approaches and contrasted their advantages and limitations with quantitative differential in-gel electrophoresis (DIGE) methods [51-53]. More recently, label-free LC-MS/MS quantification methods have been developed. These methods are typically based on determining peak-area ratios of the same peptides between different conditions. The quantitative reproducibility of these methods depends upon the peptide cluster efficiency, which is determined by the mass measurement accuracy and precision and the extreme chromatographic retention time reproducibility obtained during the experiment. It was recently discovered that a label-free approach allows accurate estimation of absolute protein concentrations in complex mixtures [54]. Using a label-free LC-MS/MS approach, a series of plasma specimens from type I Gaucher patients prior and after therapy were studied [55]. 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 [55]. The absolute levels of chitotriosidase protein detected by label-free LC-MS were found to be similar to those expected based on measured enzyme activity in specimens, a finding further validating the approach. Interestingly, proportional therapy-induced changes were noted in 6 proteins belonging to
the complement and coagulation cascades [55]. In hindsight, this finding is not surprising since Gaucher patients show a low level of coagulation activation [56]. Grouping of peptides according their changes in concentrations across conditions (for example during therapy) apparently can reveal pathways like coagulation and complement activation. This is a fascinating observation, but it should be kept in mind that the dynamic range of detectable proteins is still relatively small at present. Only the more abundant plasma proteins are quantifiably detected and the challenge for the future is to develop clever plasma fractionation approaches allowing reliable detection of additional proteins.

A third proteomics approach that has already been employed in biomarker discovery for lysosomal storage diseases is based on surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). This relatively novel application of mass spectrometry combines absorption chromatography with time-of-flight mass spectrometric detection. Using different kind of surfaces, groups of proteins can be selectively bound to a particular chip. Subsequently, chips are washed several times to remove unbound molecules and other interfering substances. As in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, molecules are co-crystallized with matrix molecules and ionized using a laser. The advantage of SELDI-TOF MS over conventional techniques is the possibility to apply complex biological samples such as serum or plasma directly due to specific retention of target proteins. SELDI-TOF mass spectrometric analysis of blood from Gaucher patients using negatively charged CM10 ProteinChip® Arrays (weak cation exchange arrays) assisted the discovery of the biomarker CCL18 [18]. A peptide of 7.8 kDa was prominent in plasma of an untreated symptomatic Gaucher patient but was nearly absent in control samples. It was subsequently identified as CCL18. Given its favourable physico-chemical characteristics for mass spectrometric detection (low molecular mass, positive charge, and relatively high abundance), it was investigated whether SELDI-TOF based quantification of CCL18 in blood could be used to quickly monitor the disease. The outcome of this study was disappointing [57]. CCL18 levels in some blood samples were significantly underestimated. Apparently, limited binding capacity and sample dependent suppression of CCL18 ionization contribute strongly to the final peak intensity. SELDI-TOF MS therefore offers no reliable procedure to quantitatively monitor CCL18 levels in blood [57].

Instead of hunting for specific protein biomarkers in complex protein mixtures like plasma, it has become popular to establish distinctive plasma protein profiles. In a fast and economic manner a subproteome of plasma can be obtained by SELDI-TOF MS. This typically results in data sets with low-samples-to-variables-ratio. To avoid erroneous results due to the undersampling, thorough statistical validation of discrimination models is crucial as was recently illustrated [58]. 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 90%. Permutation and double cross-validation proved to be crucial to avoid erroneous results 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 10 polypeptides contributing to the discrimination between the normal and Gaucher plasma subproteomes, it was found that they were all relatively small (molecular masses below 10000 Da) and up-regulated in Gaucher patients [58]. It is known that various proteases, particularly cathepsins, are elevated in Gaucher plasma [20]. This conceivably leads to unique low molecular mass degradation products. It seems unlikely that among these peptides specific biomarkers will be identified.

**Concluding remarks**

Different classes of biomarkers have to be considered for lysosomal storage disorders [59]. The first category exists of the accumulating metabolites or their secondary products thereof. The second category consists of proteins specifically secreted by storage cells themselves or by stimulated surrounding cells. A third category of less specific markers consists of lysosomal proteins more generally released into the circulation following lysosomal dysfunction [60]. Research on Gaucher disease has led to the identification of very specific protein biomarkers of storage cells in plasma of patients. Both plasma chitotriosidase and CCL18 are found to correlate with Gaucher cells and disease manifestation. Measurement of their plasma levels offers additional tools for clinicians in decision making during patient management.

For other lysosomal storage disorders genuine protein biomarkers of storage cells are unfortunately still lacking. In those disorders in which macrophages participate in accumulation of storage material, plasma chitotriosidase tends to be elevated [61-63]. Analysis of plasma or urine samples with respect to the storage compound or metabolites thereof may offer an alternative to secretory protein-based biomarkers. The efficacy of therapeutic intervention of Fabry disease and Mucopolysaccharidosis type I is presently analyzed by monitoring the levels of storage products (ceramidetrihexoside and glycosaminoglycans, respectively). Detailed investigations have to clarify to which extent these metabolite abnormalities reflect storage in tissues well enough and thus can serve as reliable biomarkers.

Novel analytical methods will increasingly allow identification of disease-related abnormalities at protein and metabolite level. Only few of them will prove to be valuable as genuine biomarkers. It is very well conceivable that some detected abnormalities will render important new insights in pathophysiological mechanisms and in the metabolic adaptations that occur in chronically diseased individuals. The expanding information at protein and metabolite level will possibly even provide novel targets for therapeutic interventions.

**Acknowledgments**

The authors wish to thank their colleagues in the clinical departments and research
laboratories in the Academic Medical Center focusing on lysosomal storage disorders. Acknowledged is the continuous support by the Gaucher and Fabry disease patient societies of the Netherlands.

References


Chapter 13


