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.
Lysosomal storage disorders are characterized by dysfunction of lysosomal processes, most commonly due to deficiencies in specific lysosomal enzymes. Without sufficient activity of a lysosomal enzyme, accumulation of corresponding macromolecular substrates takes place in the lysosomes of cell types involved in breakdown of these molecules. For many lysosomal storage disorders there exists no clear genotype-phenotype correlation and there is a striking variability in the severity of symptoms and complications between patients, sometimes even within the same family. Biomarkers are of great value for the clinical management of lysosomal storage diseases. Ideally, biomarkers originate from the pathological storage cells and are detectable in bodily fluids that can be conveniently obtained, such as blood and urine. This thesis deals with our search for biomarkers for Gaucher and Fabry disease and describes investigations showing that biomarkers indeed can support diagnosis and assist clinicians in decision making regarding the need for initiation as well as optimization of therapy.

The introduction of proteomics, a platform of mass spectrometric (MS) methods suitable for the detection and identification of proteins, has opened up new research avenues. Several proteomics techniques for studying plasma proteins have been developed in recent years. The principal aim of the research described in this thesis was to identify and characterize biomarkers for Gaucher and Fabry disease using one of these novel technologies, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). This relatively new application of mass spectrometry combines absorption chromatography with time-of-flight mass spectrometric detection. The outcome of the various investigations conducted for this thesis work is here summarized.

Biomarkers for Gaucher disease

In chapter 3 we describe an investigation regarding the value of CCL18 as Gaucher cell marker in symptomatic Gaucher patients. We report that in plasma of symptomatic Gaucher patients the chemokine CCL18 is on average 29 fold elevated, without overlap between patients’ and control values. Plasma CCL18 concentrations decrease during therapy, comparably to chitotriosidase. Immunohistochemistry demonstrates that Gaucher cells are the prominent source of CCL18. The plasma CCL18 level can serve as alternative surrogate marker for Gaucher disease and is especially useful for monitoring chitotriosidase deficient patients. Additionally, monitoring of plasma CCL18 levels proves to be very useful in determination of therapeutic efficacy.

In chapter 4, we describe a study to investigate whether SELDI-TOF MS offers a reliable procedure to quantitatively measure CCL18 levels in blood and can be used to monitor disease status of Gaucher patients. Given its low molecular mass, positive charge, and relatively high abundance, CCL18 seems a particular attractive protein for SELDI-TOF based quantitation. Therefore, we determined CCL18 levels in plasma using SELDI-TOF
MS and ELISA, in parallel. CCL18 levels in some blood samples were significantly underestimated when determined by SELDI-TOF MS. Spiking of recombinant CCL18 indicated that its detection by SELDI-TOF MS is strongly determined by the nature of the sample, even markedly varying between samples obtained from one donor taken at different time points. Independent of the total CCL18 concentration in blood only 1-10% of the chemokine bound to the ProteinChip® Array. Even when comparable amounts of CCL18 from distinct samples were bound to the ProteinChip® Array, diverse peak intensities could be observed. Thus, limited binding capacity and sample-dependent suppression of CCL18 ionization both contribute significantly to the final peak intensity. In conclusion, SELDI-TOF MS does not offer a reliable procedure to quantitatively monitor CCL18 levels in blood and thus cannot be applied in evaluation of disease status of Gaucher patients.

Chapter 5 describes the search for a biomarker for skeletal disease in Gaucher patients. Skeletal disease is one of the most debilitating symptoms for Gaucher patients. Monitoring the outcome of therapy with regard to skeletal status of Gaucher patients is problematic since currently available imaging techniques are expensive and not widely accessible. The availability of a blood test that relates to skeletal manifestations would be very valuable. We here report that macrophage inflammatory protein (MIP)-1α and MIP-1β, both implicated in skeletal complications in multiple myeloma, are significantly elevated in plasma of Gaucher patients. The increase in plasma MIP-1β levels of Gaucher patients is associated with skeletal disease. The plasma levels of both chemokines decrease upon effective therapy. Lack of reduction of plasma MIP-1β below 85 pg/mL during 5 years of therapy was observed in patients with ongoing skeletal disease. In conclusion, MIP-1α and MIP-1β are elevated in plasma of Gaucher patients and remaining high levels of MIP-1β during therapy seem associated with ongoing skeletal disease.

In chapter 6 we describe the effect of differences in therapeutic enzyme dosing regime on plasma MIP-1β concentration. Given the debate whether high dose enzyme replacement therapy (ERT) results in a faster and better response in bone we investigated whether a higher dosing regimen also leads to a superior plasma MIP-1β response. For this purpose we retrospectively determined MIP-1β responses in two comparable patient groups receiving either a relatively low dose or a relatively high dose of ERT. Plasma MIP-1β levels improved faster during the first year of treatment in the higher-dosed patient group. This was also observed for responses in chitotriosidase and bone marrow involvement.

In chapter 7 we describe the effect of therapy on the clinical and biochemical parameters of three siblings with Gaucher type III. The siblings were born between 1992 and 2004. During these years new insights regarding therapy for Gaucher type III have changed clinical practice. The two eldest siblings received ERT from the age of 24 and 6 months, respectively. The dosage was subsequently increased and ERT was combined with
substrate reduction therapy (SRT) at the age of 12 and 8 years, respectively. In the youngest sibling both high-dose ERT and SRT were initiated five months after birth. The two eldest siblings have significant neurological impairment since the age of 1.5 years, starting with a convergent strabismus (eyes not properly aligned with each other) and partial oculomotor apraxia (difficulty in controlling horizontal eye movements), later followed by cognitive decline and abnormalities concerning brain function and hearing. The neurological course in the youngest sibling is significantly better. At the age of three years, cognitive development, brain function and hearing are normal. The disturbed saccadic eye movements, observed soon after birth, slightly improved over time. Based on these results, a combined use of high-dose ERT and SRT can be regarded as a promising therapy for Gaucher type III, especially when started at a young age. Further follow-up studies are necessary to explore the long-term therapeutic effects.

Chapter 8 describes our proteomic analysis of Gaucher disease using classical 2D gel electrophoresis. Plasma of Gaucher disease type I patients was compared with plasma of healthy volunteers. In Gaucher plasma, several abundant proteins with a high molecular weight were absent, while a new group of low molecular weight proteins appeared. These small proteins were identified as degradation products due to proteolysis, which could be completely inhibited by using thiourea/urea in the rehydration buffer instead of classical ‘urea only’. Mixing Gaucher plasma with control plasma demonstrated that breakdown was due to the presence of (an) active protease(s) in Gaucher plasma. Our observations can be explained by partial protein denaturation. Whereas the protease substrates are denatured under ‘urea only’ conditions, the responsible protease(s) seem unaffected. Therapy results in disappearance of extensive proteolysis in Gaucher plasma presumably due to a drop in protease levels. Incubation with Concanavalin A Sepharose™ resulted in partial inhibition of proteolytic activity in Gaucher plasma, suggesting that (some of) the protease(s) responsible for breakdown may be glycoproteins. Reduction of proteolysis by different protease inhibitors shows that a mixture of proteases, which are excessively present in plasma of symptomatic Gaucher patients, is responsible for the intriguing massive protein breakdown observed with 2DGE. Thus, our study revealed induction of large-scale proteolysis in Gaucher plasma ‘in vitro’, the extent of which seems to correlate with disease severity.

In chapter 9 we present a strategy for the statistical validation of discrimination models in proteomics studies. Several existing tools are combined to form a solid statistical basis for biomarker discovery that should precede a biochemical validation of any biomarker. These tools consist of permutation tests, single and double-cross validation. As a classification method, Principal Component Discriminant Analysis (PCDA) is used; however, the methodology can be used with any classifier. Cross-validation of PCDA can easily be combined with a new variable selection method, called rank products. The strategy is especially suited for the low-samples-to-variables-ratio (undersampling) case, as often encountered in proteomics and metabolomics studies. A data set containing serum
samples from Gaucher patients and healthy controls was used as a test case. Double cross-validation shows that the sensitivity of the model is 89% and the specificity 90%. Potential putative biomarkers are identified using the novel variable selection method. Results from permutation tests show that even single cross-validation does not guarantee unbiased results, supporting the choice of double cross-validation as the tool for determining error rates when modelling procedures involve a tuneable parameter. The validation of discrimination models with a combination of permutation tests and double cross-validation helps to avoid erroneous results, which otherwise may arise due to undersampling.

In chapter 10 we present a sensitive method to detect point mutations in proteins from complex samples. The method is based on SELDI-TOF MS but can be extended to other MS platforms. The target protein in this study is the lysosomal enzyme glucocerebrosidase, the key enzyme in Gaucher disease. Deficiency of glucocerebrosidase activity results in accumulation of glucosylceramide in macrophages. The relationship between glucocerebrosidase genotypes and Gaucher patient phenotypes is not strict. The possibility to measure protein levels of glucocerebrosidase in clinical samples may provide deeper insight with regard to the genotype-phenotype relationship. To this end glucocerebrosidase was isolated in a one-step enrichment step through interaction with an immobilized monoclonal antibody (8E4). After on-chip digestion of the antibody-antigen complex with trypsin, a total of 25 glucocerebrosidase peptides were identified (sequence coverage ~60%), including several peptides containing mutated amino acid residues. The described methodology allows mutational analysis at the protein level, directly measured in complex biological samples without the necessity of elaborate purification procedures.

Biomarkers for Fabry disease
In chapter 11 an extensive investigation into the clinical manifestations of Dutch Fabry patients is presented. Clinical and biochemical characteristics of 96 (25 deceased) Dutch Fabry patients were collected retrospectively; all before initiation of enzyme therapy. Analysis of the characteristics of the Dutch Fabry cohort revealed a limited relationship between various disease manifestations. Additionally, individual symptoms do not correlate with elevated urinary or plasma Gb3 levels, limiting their value as surrogate disease markers.

In chapter 12 we describe our search for a blood biomarker that reliably reflects the clinical manifestation of Fabry disease. For this purpose, we compared serum of controls and Fabry patients using SELDI-TOF MS. We recently have demonstrated that principal component discriminant analysis (PCDA) of SELDI-TOF MS data obtained from serum specimens allowed classification of Gaucher disease patients. We here report on the value of SELDI-TOF MS serum profiling for discrimination of symptomatic Fabry patients using PCDA and support vector machines (SVM) analysis. It is much harder to distinguish between Fabry patients and controls than between Gaucher patients and controls. The differences between the Fabry and control spectra are probably much smaller than the differences between the Gaucher and control spectra.
Finally, in **addendum 1**, our search for a biomarker for delirium is presented. For this purpose plasma and serum protein profiles in patients with and without postoperative delirium were compared. Serum and plasma of patients aged 65 years or more, that were admitted for surgery following a hip fracture, were used. Protein profiles were generated by SELDI-TOF using CM10 and Q10 ProteinChip® Arrays. The largest difference was found in EDTA plasma using CM10 ProteinChip® Arrays, which was confirmed in the validation group. Taking both groups together, three discriminating peaks were found in delirious patients. These peaks presumably correspond to (forms of) hemoglobin-β.

Summarizing, research on Gaucher disease type I has led to the identification of two new biomarkers of storage cells in plasma of patients. The first new biomarker, CCL18, was found to correlate with Gaucher cells and disease manifestation, just like plasma chitotriosidase. Measurement of plasma CCL18 levels offers an additional tool for clinicians in decision making during patient management and is especially useful for monitoring chitotriosidase deficient individuals. 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. In addition, we found markedly elevated levels of the chemokine MIP-1β in plasma of symptomatic Gaucher patients. Interestingly, this protein was found to be produced by surrounding inflammatory cells and not by mature Gaucher cells, as is the case for chitotriosidase and CCL18. A lack in response in plasma MIP-1β upon ERT was found to correlate with ongoing skeletal disease. Correction in plasma MIP-1β is dose dependent during the initial phase of enzyme replacement therapy of Gaucher disease, suggesting that not only the initial correction of Gaucher cells but also that of associated phagocytes is enzyme dose-dependent.

For Fabry disease genuine protein biomarkers are still lacking. Analysis of the clinical and biochemical characteristics of the Dutch Fabry patients did not yield a (possible) biomarker. We also searched for biomarkers of Fabry disease using mass spectrometry. Our SELDI-TOF MS protein profiling, unfortunately, gave no reliable discrimination between symptomatic Fabry patients and healthy controls. In hindsight, the result of our investigations is not so surprising since no single blood biomarker for Fabry disease has so far been detected. It appears that in contrast to what was thought previously, lipid-laden endothelial cells of Fabry patients are not grossly abnormal in behaviour and function, and are not releasing specific proteins into the circulation that are detectable by plasma protein profiling with the currently available SELDI-TOF MS methodology. Of note, the same methodology could be successfully employed to discriminate serum specimens from Gaucher patients and healthy subjects.
Discussion

Biomarkers can offer important information for clinical decision-making regarding diagnosis, determination of disease severity, initiation of therapy, monitoring of therapeutic efficacy and optimizing of therapy regimens for individual patients. During our search for biomarkers for Gaucher and Fabry disease, novel insights were obtained (for a review, see chapter 13). Some aspects of these biomarkers and their associated diseases are still not well understood and limitations of present biomarkers have become apparent. In this chapter a number of selected topics are discussed and suggestions for future studies on (biomarkers for) lysosomal storage disorders are proposed.

Section I: Biomarkers for Gaucher disease

Research on Gaucher disease has led to the identification of very specific protein biomarkers for the pathological lipid-laden macrophages (Gaucher cells) that are elevated in plasma of patients. Chitotriosidase [1-3] and CCL18 [4,5] are produced by Gaucher cells and secreted into the circulation. Plasma levels of both chitotriosidase and CCL18 correlate to some extent with disease manifestations such as liver and spleen enlargement and thrombocytopenia [1,5,6]. Their measurement offers additional tools for clinicians in decision making during patient management. Plasma chitotriosidase, being about 1000-fold elevated in Gaucher patients, provides an excellent indicator of overall Gaucher cell burden and a very sensitive tool to follow disease activity. In patients that are chitotriosidase deficient, monitoring of plasma CCL18 is a good and reliable alternative. Plasma chitotriosidase and CCL18 do not reflect one particular clinical symptom of Gaucher disease since they stem from Gaucher cells at various body locations [1]. As the degree of skeletal involvement does not correlate with total Gaucher cell burden, the two biomarkers appear of limited use to assess skeletal disease. Since skeletal disease is one of the most debilitating symptoms for Gaucher patients, the availability of a blood test that relates to skeletal manifestations would be very valuable. Conventional markers of osteoclast and osteoblast activity prove to be not very informative for assessment of skeletal disease in Gaucher patients [7,8]. In chapter 5 [8] we show that macrophage inflammatory protein (MIP)-1α and MIP-1β, both implicated in skeletal complications in multiple myeloma, are significantly elevated in plasma of Gaucher patients. A correlation was observed between plasma MIP-1β and extent of skeletal disease. A lack of reduction of plasma MIP-1β below 85 pg/mL during 5 years of therapy was observed in patients with ongoing skeletal disease. 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. Moreover, since it has become clear that skeletal disease is more difficult to treat and control by enzyme replacement therapy (ERT) than other clinical symptoms in Gaucher disease, it is of major importance to recognize it in its earliest stages and to monitor accurately its progression. An important future research question is thus whether plasma MIP-1β is suitable for this purpose. If not, research should proceed to find a marker more closely correlating with skeletal disease,
which may be useful in preventing bone complications in the future. As long as a convenient bone specific biochemical marker is lacking, adequate evaluation of skeletal disease in Gaucher patients will have to solely rely on radiology. A correlation between the infiltration of bone marrow by Gaucher cells and skeletal complications has been documented [9,10]. Unfortunately, the most suitable imaging technique for this purpose, QCSI (quantitative chemical shift imaging), is only available in few centres and therefore less sensitive alternatives have to generally employed [9,10]. With regard to skeletal disease, special attention should be paid to patients that are splenectomized, and manifested already bone complications, since these appear to be at risk for further osseous complications. It has to be carefully comparatively established whether a lack or slow improvement during ERT in the correction of bone marrow as measured by QCSI, chitotriosidase and MIP-1β or a combination of these offers prognostic information regarding skeletal disease [11].

Section II: Biomarkers for Fabry disease

Despite the fact that Fabry disease and Gaucher disease are both caused by defects in lysosomal glycosidases degrading glycosphingolipids, their clinical manifestation is remarkably different. This difference is usually ascribed to the fact that in Fabry disease storage of globotriasylceramide (Gb3) occurs in multiple cell types, particularly in endothelial cells, whereas in Gaucher disease exclusively tissue macrophages develop into storage cells. In contrast to the situation for Gaucher disease, genuine protein biomarkers of storage cells in Fabry disease are unfortunately still lacking. So far only modest abnormalities in plasma chitotriosidase [12] and myeloperoxidase [13] have been consistently observed, pointing to the involvement of macrophages and leukocytes in the pathophysiology of Fabry disease. Their application as biomarkers may be however very limited given the considerable overlap in levels encountered in Fabry patients and normal subjects.

Accumulating Gb3 has obviously also been considered as a biomarker for Fabry disease. In chapter 11 [14] we investigated the clinical value of urinary or plasma Gb3 levels as marker for Fabry disease manifestation. To our disappointment we observed that none of the Fabry related symptoms correlated well with urinary or plasma Gb3 levels. In addition, elevated levels of Gb3 in plasma or urine did not correlate with severity of disease (MSSI). Prominent Gb3 accumulation occurs in hemizygotes at or even before birth, long before any clinical symptoms develop [15]. The discrepancy between early storage of Gb3 and clinical symptoms is also noted in Fabry mice generated by disruption of the α-galactosidase A gene [16]. Thus, the clinical value of urinary or plasma Gb3 as biomarker for manifestation of Fabry disease seems very limited. Other investigators have come to a similar conclusion [17,18]. The very recent demonstration of dramatically elevated levels of lysoglobotriaosylphosphoglycan in plasma and tissue of Fabry patients as well as mice with a disrupted α-galactosidase A gene, prompts further investigations in its value as prognostic marker and possible role in pathogenesis [19].

In chapter 12 we used surface-enhanced laser desorption/ionization time-of-flight mass...
spectrometry (SELDI-TOF MS) to investigate whether serum contains proteins specifically secreted by storage cells themselves (or by stimulated surrounding cells). Our study failed to detect useful discriminatory differences between Fabry and control SELDI-TOF MS serum profiles. In retrospect, these negative results are not so surprising since no single blood biomarker for Fabry disease has been detected so far [20]. In Fabry disease, storage of Gb3 in arterial walls is thought to underlie the clinical manifestations of the disease [21]. However, directed searches in plasma or serum of Fabry patients for markers of coagulation activation, fibrinolysis, platelet activation and endothelial activation have given negative results. Vedder et al. [22] showed only minimal abnormalities in coagulation activation, fibrinolysis, platelet activation and endothelial activation in patients with Fabry disease, except in the more severely affected patients with renal impairment. Vedder et al. conclude that the reported abnormalities are probably better explained by the renal insufficiency than by Fabry disease itself. It is of particular interest to note that no significant abnormalities were detected in Fabry plasma specimens regarding von Willebrand factor or endothelial cell derived microparticles, phenomena usually associated with endothelial cell activation. Thus, in contrast to the still common belief [21], lipid-laden endothelial cells of Fabry patients seem not grossly abnormal in behavior and function. This may explain our inability to detect abnormal concentrations of endothelial cell derived proteins during Fabry serum protein profiling with the SELDI-TOF MS methodology. The question however still remains whether other (analytical) methods will not be able to detect disease-related abnormalities in body fluids of Fabry patients such as blood and urine. Further efforts should therefore be undertaken to establish this (see below).

**Limitations of SELDI-TOF MS**

A proteomics approach employed in biomarker discovery is SELDI-TOF MS. This relatively novel application of mass spectrometry combines absorption chromatography with time-of-flight mass spectrometric detection. The advantage of SELDI-TOF MS over conventional techniques is the possibility to apply complex biological samples such as serum or plasma directly because of the specific retention of a certain class of target proteins only. Given CCL18’s low molecular mass, positive charge, and relatively high abundance, this chemokine seems a particular attractive protein for SELDI-TOF based quantification. Therefore, we determined CCL18 levels in plasma using SELDI-TOF MS and ELISA in parallel, and investigated whether SELDI-TOF MS based quantification of CCL18 in blood can be used to quickly monitor Gaucher disease. The outcome of this study, described in chapter 4 [23], was rather disappointing. 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 does not seem to offer a reliable procedure to quantitatively monitor CCL18 levels in blood. It is highly likely that similar problems occur with the detection of other proteins in blood samples when analyzed by SELDI-TOF MS. Thus, our study is of more general interest, as it demonstrates that semi-quantitative monitoring of
disease-specific biomarkers in complex fluids, such as blood, by SELDI-TOF MS still remains highly problematic.

Despite this, analyzing blood proteins by means of SELDI-TOF MS has become a popular approach to obtain disease-specific protein profiles. Instead of looking for specific protein biomarkers in complex protein mixtures like plasma or serum, mass spectra of samples of diseased and (healthy) control individuals are measured with the objective of distinguishing between the control and diseased groups. Data analysis methods are used to compare the protein profiles and create classification models. Since data sets typically have a low-samples-to-variables-ratio, thorough statistical validation of discrimination models is crucial. We demonstrated in chapter 9 [24] that principal component discriminant analysis of SELDI-TOF MS data obtained from serum specimens allowed classification of Gaucher disease patients. Although this approach was successful for Gaucher disease, a comparable study failed to detect useful discriminatory differences between Fabry and control SELDI-TOF MS serum profiles. As mentioned earlier, this is probably not only due to limitations of the technique, but intrinsic to the pathophysiology of Fabry disease. Thus, the use of SELDI-TOF MS to distinguish between control and diseased groups, based on complete protein profiles, remains a promising application of the technique (bearing in mind the fact that Gaucher disease samples are exceptional in the levels and variety of protein differences when compared to control samples). However, although many samples can be prepared for mass spectrometric analysis in only a few hours, one has to keep in mind that statistical validation of the discrimination models is indispensable. Besides biochemical and clinical knowledge, expertise with statistical validation is thus essential to determine the statistical and predictive value of candidate biomarkers.

Suggestions for future studies

We reported elevated MIP-1α and MIP-1β levels as newly detected plasma abnormalities in Gaucher patients. In particular insufficient correction in plasma MIP-1β following therapy seems associated with ongoing skeletal disease. Further research with larger groups of well-documented Gaucher patients will have to reveal whether plasma MIP-1β levels can be of additional value in clinical management of Gaucher patients, particularly for the management and prediction of their skeletal disease. Moreover, additional studies are clearly necessary to elucidate the pathophysiology of skeletal problems in Gaucher patients. The development of guidelines to monitor and treat bone complications in Gaucher patients will remain difficult as long as the pathophysiology of Gaucher skeletal disease is incompletely understood. Since no clear-cut indications for classical osteoporosis have been firmly documented [7], it is suggested that special molecular mechanisms are involved in the skeletal disease in Gaucher patients. An important research question regarding skeletal disease in Gaucher patients is whether MIP-1β directly underlies disease processes in the bone marrow. MIP-1α and MIP-1β have already been implicated in the pathogenesis of skeletal disease in patients suffering from multiple myeloma [25,26]. It is possible that, amongst other factors, chemokines like MIP-1β play...
Discussion

an important role in the disturbed balance of bone resorption and formation in Gaucher patients. The recent availability of suitable Gaucher mouse models should allow such investigations [27]. Our present study (chapter 5 [8]) did not address the relationship between plasma MIP-1β levels and localized osteolysis or generalized osteopenia/osteoporosis. Future investigations should address these possible relationships. Since most skeletal complications are practically irreversible it is of great importance to be able to monitor skeletal disease in Gaucher patients in such a way that skeletal complications can be prevented in the future.

With respect to the evaluation of disease progression and treatment response in Fabry patients, further efforts should be undertaken to identify biomarkers. This is a challenging research area because the disease is not well understood. A subgroup of Fabry patients has benefited little from ERT. It appears that treatment has been started too late in the disease process. The absence of a biomarker hampers good monitoring of these patients. Presently, organ function is the best parameter to monitor treatment efficacy. Thus, future (laboratory and clinical) investigations are required to study the aetiology and course of Fabry disease and identify possible biomarkers. The recent availability of a suitable Fabry mouse model in principle should allow rapid progress in such investigations [16]. An approach that also deserves special attention since it has been recently employed in a search for plasma biomarkers in a lysosomal storage disorder is a label-free LC-MS/MS quantification method. This method is based on determining peak-area ratios of the same peptides between different conditions. It was recently discovered that this approach allows accurate estimation of absolute protein concentrations in complex mixtures [28]. Using LCMS5, a series of plasma specimens from type I Gaucher patients before and after therapeutic intervention were studied [29]. Marked therapy-induced differences were found 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 [29]. Investigations should be undertaken to test whether this approach is also successful in the case of Fabry disease.

In summary, although considerable progress has been made in understanding Gaucher and Fabry disease, many issues still need to be clarified. In the light of this thesis, the mechanism underlying the skeletal disease in Gaucher patients as well as the identification of further 'bone' biomarkers are of particular interest. In addition, a genuine biomarker for Fabry disease would be of great value for monitoring therapeutic efficacy.

References

Chapter 14


[23] M.J. van Breemen, B. Bleijlevens, C.G. de Koster, J.M.F.G. Aerts, Limitations in quantification of the


