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Chapter Two

Identification and use of biomarkers in Gaucher disease and other lysosomal storage diseases

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Abstract

The value of biomarkers in the clinical management of lysosomal storage diseases is best illustrated by the present use of plasma chitotriosidase levels in the diagnosis and monitoring of Gaucher disease. The enzyme chitotriosidase is specifically produced and secreted by the pathological storage macrophages (Gaucher cells). Plasma chitotriosidase levels are elevated on average 1000-fold in symptomatic patients with Gaucher disease and reflect the body burden on storage cells. Changes in plasma chitotriosidase reflect changes in clinical symptoms. Monitoring of plasma chitotriosidase levels is nowadays commonly used in decision making regarding initiation and optimization of costly therapeutic interventions (enzyme replacement therapy or substrate reduction therapy). A novel substrate has been developed that further facilitates the measurement of chitotriosidase in plasma samples. Moreover, an alternative Gaucher-cell marker, CCL18, has been very recently identified and can also be employed to monitor the disease, particularly in those patients lacking chitotriosidase due to a genetic mutation. There is a need for comparable surrogate markers for other lysosomal storage diseases and the search for such molecules is an area of intense investigation.

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Biomarkers

Biomarkers are characteristics that are measured and evaluated as indicators of normal or pathological processes, or responses to a therapeutic intervention. When the relationship between a biomarker and a clinically relevant disease feature is established, the biomarker can serve as a surrogate endpoint to substitute for a clinical endpoint. Biomarkers are potentially of great value for the clinical management of lysosomal storage diseases (LSDs). Ideally, biomarkers originate from the pathological storage cells and are detectable in bodily fluids that can be conveniently obtained, such as blood and urine. As illustrated below by the example of Gaucher disease, true biomarkers may indeed support diagnosis and may assist clinicians in decision making regarding the need for the initiation of therapy as well as the optimization of therapy [1].

Biomarkers for Gaucher disease

Gaucher disease is the most common LSD worldwide. This recessively inherited disease is caused by a deficiency in the lysosomal hydrolase glucocerebrosidase (EC 3.2.1.45), resulting in massive intralysosomal storage of the natural glycosphingolipid glucosylceramide [2]. In the majority of patients with Gaucher disease (those suffering from the so-called type I variant), lipid storage is restricted to tissue macrophages. These storage cells (named Gaucher cells) are viable macrophages, which secrete various factors that promote local tissue destruction and ongoing formation of storage cells. Characteristic features of Gaucher disease are hepatosplenomegaly, pancytopaenia and skeletal deterioration. These pathological entities are attributed to the accumulation of Gaucher cells in the spleen, liver and bone marrow [2].

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 and lysozyme in serum samples of patients with Gaucher disease have been documented for some time (for a review see [3]). More recently, increased plasma levels of various cathepsins, for example cathepsin K, have been reported in patients with Gaucher disease [4]. All of 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 concentrations in the serum of symptomatic patients with Gaucher disease may overlap with those 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 that led to the discovery of a very marked abnormality in the serum of symptomatic patients with Gaucher disease. Serum from such individuals showed a 1000-fold increased capacity to degrade the fluorogenic substrate 4-methylumbelliferyl-chitotrioside [5]. The corresponding enzyme had not been previously described and was named chitotriosidase. The chitotriosidase protein was subsequently purified and its cDNA was cloned [6,7]. Chitotriosidase was found to be the human analogue of chitinases from lower organisms. *In situ* hybridization and histochemistry of bone marrow aspirates and

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sections of spleen from patients with Gaucher disease revealed that chitotriosidase is very specifically produced by storage cells. This is also supported by the close linear relationship between levels of chitotriosidase and glucosylceramide in different sections of spleen from patients with Gaucher disease. As glucosylceramide is the best possible quantitative measure for storage cells, it may be deduced that chitotriosidase production is directly proportional to the number of Gaucher cells. In a culture model of Gaucher cells, chitotriosidase accounts for almost 10% of the total level of secreted protein. In sharp contrast, common tissue macrophages and dendritic cells do not produce chitotriosidase. These observations help to explain the very specific, gross elevation in chitotriosidase levels in the blood of patients with Gaucher disease. A relationship between the total body burden on storage cells in patients with Gaucher disease 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 the levels of enzyme secreted by Gaucher cells in various body locations [5].

The activity of chitotriosidase in plasma can be determined by monitoring the hydrolysis of 4-methylumbelliferyl-chitotrioside. However, the ability of chitotriosidase to transglycosylate as well as hydrolyze this substrate complicates the enzyme assay. 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 method is to measure the activity of chitotriosidase with respect to the recently designed fluorogenic substrate, 4-methylumbelliferyl deoxychitotrioside [8].

Interpretation of plasma chitotriosidase levels is intrinsically complicated by the common occurrence of a particular 24-base-pair duplication in the chitotriosidase gene, which prevents the formation of chitotriosidase protein [9]. In most ethnic groups, about one in three individuals carries this abnormality and about 1 in 20 individuals is homozygous for this trait. The frequency of chitotriosidase deficiency is similar among patients with Gaucher disease [4,9]. The severity and progression of disease in such patients cannot, therefore, be monitored by measuring chitotriosidase activity in plasma.

Very recently, a proteomics and genomics search led us to the discovery that Gaucher cells massively overproduce and secrete the chemokine CCL18 (also known as pulmonary and activation-regulated chemokine [PARC]) [10]. Plasma CCL18 levels are 10- to 50-fold elevated in symptomatic patients with Gaucher disease. Measurement of plasma CCL18 levels is, therefore, an excellent additional tool for monitoring changes in the body burden of Gaucher cells. It is particularly useful for the evaluation of those patients that are deficient in chitotriosidase.

The use of biomarkers for monitoring therapy for LSDs

Prior to the 1990s, the only available therapy for Gaucher disease was bone marrow transplantation. Retrospective analysis of plasma samples from patients with Gaucher disease who had undergone successful bone marrow transplantation revealed a gradual reduction in plasma chitotriosidase levels, coinciding with the disappearance of storage cells [11]. The considerable risks associated with transplantation, however, have limited its

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application. The pioneering studies by Brady, Barranger and co-workers at the National Institutes of Health in Bethesda, USA have led to the development of a broadly applicable therapy for type I Gaucher disease (for an overview see [12]). This treatment, generally referred to as enzyme replacement therapy (ERT), is based on intravenous infusions of a human glucocerebrosidase preparation. The enzyme, nowadays the recombinant product imiglucerase (Cerezyme®; Genzyme Corp.), is modified in its *N*-linked glycans to favour uptake by mannose receptors. Such receptors are abundant on the surfaces of tissue macrophages and mediate endocytotic delivery of their ligands to lysosomes. ERT usually results in profound corrections in organomegaly and haematological abnormalities in patients with Gaucher disease [13]. Stabilization of skeletal disease and reversal of bone marrow infiltration occurs in most patients. The changes in plasma chitotriosidase levels in response to ERT have been extensively monitored, and a major reduction in levels of the biomarker is usually observed. However, an extensive study in the Academic Medical Center in Amsterdam revealed that a lack of response of plasma chitotriosidase levels to ERT is always accompanied by a lack of clinical response. In contrast, a prominent reduction in levels of the biomarker precedes significant clinical improvements [1]. Changes in concentrations of plasma CCL18 are proportional to those in plasma chitotriosidase during the successful treatment of Gaucher disease [10]. This further substantiates that the source of both biomarkers is Gaucher cells.

An alternative treatment for Gaucher disease has recently been developed; so-called substrate reduction therapy. As first described by Radin in 1996, it is hypothesized that a partial inhibition of glycosphingolipid synthesis may balance the reduced catabolic capacity in patients with Gaucher disease [14]. The target for intervention is the enzyme glucosylceramide synthase, which catalyzes the formation of glucosylceramide from ceramide and uridine diphosphate-glucose. An important breakthrough was the realization that oral administration of the small compound *N*-butyl-deoxynojirimycin (Zavesca®; Actelion) can inhibit glycosphingolipid biosynthesis without overt side effects [15]. A multi-center clinical study revealed that oral administration of 100 mg *N*-butyl deoxynojirimycin three times daily led to significant improvements in organomegaly, haematological abnormalities and radiological abnormalities in spinal bone marrow [16]. These findings have led to the licensing of Zavesca® for treatment of moderate type I Gaucher disease in adult patients who are unsuited to ERT [17]. Concomitant with clinical improvements in patients receiving *N*-butyl-deoxynojirimycin, clear reductions in plasma chitotriosidase and CCL18 levels have been noted [10].

The use of biomarkers in establishing guidelines for treatment

Thorough retrospective analysis of corrections in levels of chitotriosidase and clinical improvements in patients with Gaucher disease receiving ERT has led to the implementation of the following guidelines in The Netherlands: in patients in whom the initiation of treatment is questionable based solely on clinical parameters, a chitotriosidase activity above 15 000 nmol/hour/mL in the case of individuals with normal chitotriosidase genotype, and an enzyme activity above 7500 nmol/hour/mL in the case of individuals

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heterozygous for the chitotriosidase mutation, serves as an indicator of a high Gaucher cell burden and is a criterion for the initiation of treatment. A reduction in chitotriosidase activity of less than 15% after 12 months of treatment should be a reason to consider a dose increase. Furthermore, a sustained increase in chitotriosidase activity at any point during treatment should alert the physician to the possibility of clinical deterioration and the need for adjustment of therapy [1].

Biomarkers for other LSDs

The successful development of therapeutic interventions for type I Gaucher disease based on ERT and substrate reduction therapy has resulted in investigations of comparable approaches for the treatment of other LSDs. Meanwhile, recombinant enzyme preparations are registered for the treatment of Fabry disease (agalsidase alfa [Replagal®; Shire] and agalsidase beta [Fabrazyme®; Genzyme Corp.]) and mucopolysaccharidosis type I (Aldurazyme®, Genzyme Corp.). Drugs are currently being developed for other inherited LSDs. The importance of biomarkers in the optimization of a therapeutic approach have become clear over the last decade. In view of this it is not surprising that considerable attention is focused on the detection of useful biomarkers for other LSDs. The analysis of gene expression in storage cells and/or a thorough survey of the protein composition of the bodily fluids [12] of symptomatic patients have been used to identify potential new biomarkers. The latter approach has become more feasible by the recent availability of mass spectrometric techniques that allow accurate analysis of individual proteins in complex mixtures such as plasma and urine. For example, the discovery of the biomarker CCL18 in plasma from patients with Gaucher disease by surface enhanced laser desorption ionization mass spectrometry suggests that a similar approach might also yield suitable biomarkers for some of the other LSDs [10].

It should be kept in mind that the identification of biomarkers in patients with Gaucher disease is favoured by the fact that Gaucher cells occur in very large numbers and that these cells actively secrete proteins. In this regard it should also be mentioned that analysis of plasma or urine samples with respect to the storage compound or metabolites thereof might offer an alternative to secretory protein-based biomarkers. For example, the efficacy of treatment in Fabry disease and mucopolysaccharidosis type I is presently analyzed by monitoring the levels of storage products (the lipid globotriaosylceramide and glucosaminoglycans, respectively). Detailed investigations must clarify to what extent these metabolite abnormalities reflect the actual intralysosomal storage in tissues. Only careful comparisons of changes in clinical parameters with those in candidate markers will help to establish their value as biomarkers that allow prediction of clinical benefit or risk [18].

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