Plasma Glucosylsphingosine: A Specific and Sensitive Biomarker for the Primary Diagnostic and Follow-up in Patients with Gaucher Disease

AK Giese, U Grittner, J Lukas, D Mascher, A Mühl, S Eichler, H Mascher, A Rolfs

1 Albrecht-Kossel-Institute for Neuroregeneration, University of Rostock, Rostock, Germany, 2 Department for Biostatistics and Clinical Epidemiology, Charité University Medical Centre, Berlin, Germany, 3 pharm-analyt Labor GmbH, Baden, Austria, 4 Centogene GmbH, Rostock, Germany

Contact: anne-katrin.giese@med.uni-rostock.de

Introduction

Biomarkers play an essential role in the early detection, and monitoring of metabolic diseases, this also holds true for Lysosomal Storage Disorders (LSDs), a highly heterogeneous group of hereditary diseases where defects in genes encoding for lysosomal enzymes or transporters result in the accumulation or misdistribution of non-degraded macromolecules. Ideally a biomarker can be used for the initial diagnosis, the determination of disease severity, monitoring of the progress of the disease and evaluation of treatment. Here, we determined the sensitivity and specificity of Glucosylsphingosine for the primary diagnosis and monitoring of Gaucher disease (GD), where a defect in the beta-Glucosidase (GBA) gene leads to the accumulation of glucosylceramide. Overall, we evaluated Glucosylsphingosine by comparing healthy controls, Gaucher patients, Gaucher carriers and patients with other LSDs to determine the sensitivity and specificity of Glucosylsphingosine. The biomarker was compared to Chitotriosidase and CCL18/PARC, which both are highly elevated in a number of LSDs and reflect the burden of disease on macrophages due to accumulation of macromolecules, but are not specific for GD. In addition, Chitotriosidase levels may be normal even in GD patients due to a common 24-bp duplication in the CHIT1 gene. In addition to the evaluation of sensitivity and specificity of Glucosylsphingosine, we also assessed long-term data of 19 GD patients before and after onset of enzyme replacement therapy.

Results

Table 1: Overview of all enrolled subjects. In total 129 GD patients, 15 GD carriers, 261 patients suffering from other LSDs and 148 controls were enrolled. The gender was not distributed equally among all four subcohorts, the males being predominant in the GD carrier group (p=0.029), though this was also the smallest cohort. Age-wise there were significant differences in the four cohorts as well. The healthy control group and the GD patient group were younger than the GD carriers and patients with other LSDs (p=0.012).

Table 2: Sensitivity and specificity for different biomarkers for the diagnosis of GD.

Methods

Patients and blood sample:

Blood samples were obtained from patients enrolled by the Albrecht-Kossel-Institute for Neuroregeneration (Alkor), informed consent was obtained from all adults. The protocol of the study has been approved by the local Ethical Committee of the University Rostock. Patients undergoing therapy were treated according to standard protocols. Aside from GD patients also GD carriers, healthy controls and patients with other LSDs (Neuro-Falk Tipe C disease, Fabry disease, Pompe disease, Fabry disease and Hunter disease) were enrolled.

Biochemical and genetic analysis:

Standard analysis of GBA gene, CCL18/PARC and Chitotriosidase were performed according to standard protocols [1-2].

Method for Determination of free Glucosylsphingosine in plasma:

50 µL of the sample were mixed with 100 µL of internal Standard working solution (1000) After centrifugation at 4000 rpm for 2 minutes the clear supernatant was transferred into auto sampler vials and injected into the HRESecs-MS system. Mobile phase used for gradient elution was 50 mM formic acid in water and 50 mM formic acid in acetonitrile/acetone/toluene (1:1:0.5). HRESecs mobile was set at 0.5 mL/min on an AEX 3 OD column (OD 2.1 mm) at 60°C, injection volume was 5 µL. Retention time for the analyte was approximately 3.6 minutes and for the internal standard (lysosu-2b, sufficient amount added if needed during sample preparation) approximately 3.0 minutes. For determination of free Glucosylsphingosine in plasma the AEX 4000 MS/MS system was used for electrospray ionization in RR mode in positive mode at 500°C. For details please see Raths et al. 2013 [3].

Summary

- Glucosylsphingosine is a sensitive and specific biomarker for GD
- For the pathological cut-off of 12 ng/ml the sensitivity and specificity is 100%
- After the onset of ERT the biomarker levels lowered significantly
- We will continue to assess patients undergoing ERT to determine the correlation between disease severity and Glucosylsphingosine

More than one measurement was available. This resulted in a sample of 148 healthy controls, 13 GD patients, 129 GD patients and 261 patients with other LSDs. Main demographic data (mean age, gender) was determined for all four cohorts. The accuracy of values of the Glucosylsphingosine, Chitotriosidase, enzyme activity and CCL18/PARC in discriminate patients with GD disease from patients without GD was evaluated using Receiver Operating Characteristic (ROC) curve analysis [4-5]. The area under the curve (AUC) and the 95% confidence limits for the different biomarkers were determined. For the comparison of two biomarkers (Glucosylsphingosine vs. Chitotriosidase, Glucosylsphingosine vs. CCL18/PARC) paired sample statistical techniques were used [6]. The ROC curves were calculated using R 2.14.4, Release Version 18.02 (2-P SPSS, Inc. 2008, Chicago, IL, www.spss.com). The comparisons of ROC curves and the linear mixed models were done using SAS software, version 9.2 of the SAS System for Windows (© 2008 SAS Institute Inc., Cary, NC, USA). For the evaluation of Glucosylsphingosine after onset of ERT in GD patients, we analyzed non-aggregated data for patients for whom several blood samples were available (19 GD patients). The first measurement under therapy for every patient was defined as time point zero. Linear mixed models were used to test for a time dependent reduction when comparing values before and after initialization of ERT.

- Statistical Tests: Statistical analyses were performed using SAS software, version 9.2 of the SAS System for Windows (© 2008 SAS Institute Inc., Cary, NC, USA). The statistical analyses were done using Linear Mixed models and exact P values were computed for the significance of the differences between groups.

Figure 1: Glucosylsphingosine levels in the four subcohorts. Level of Glucosylsphingosine is illustrated in the entire cohort (A) and separated according to gender (B). Glucosylsphingosine in GD patients was compared to healthy controls, GD carriers and patients with other LSDs.

Figure 2: ROC curve analysis for comparison of Glucosylsphingosine with Chitotriosidase and CCL18/PARC. Glucosylsphingosine (A; red line, area under the curve (AUC) = 1.00) and Chitotriosidase (A; blue line; AUC = 0.96) as well as Glucosylsphingosine (B; red line; AUC = 1.00) and CCL18 (B; blue line; AUC = 0.86) to discriminate the accuracy of two values. Glucosylsphingosine is significantly more accurate than Chitotriosidase (A: p=0.027, n=228) and CCL18 (B: p=0.001, n=207).

Figure 3: Monitoring of ERT by Glucosylsphingosine. The course of the Glucosylsphingosine was determined after onset of treatment, the time point zero was defined as the first value after onset of therapy. The course for 19 GD patients undergoing ERT is shown.

Note: To convert enzyme activity to specific activity, multiply the enzyme activity by 100,000.