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

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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 heterogenous 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

	Healthy Controls		GD Carriers		GD Patients		Other LSDs	
N individuals	148		13		129		261	
%	26.9		2.4		23.4		47.4	
N measures	163		15		456		340	
Age in years:								
Median	29		35		29		38	
Interquartile Range	(5-48)		(31-59)		(8-44)		(19-50)	
Number of Cases	(n=141)		(n=13)		(n=119)		(n=238)	
	male	female	male	female	male	female	male	female
n	81	67	8	5	66	56	117	144
%	54.7	44.8	64.7	35.3	52.2	47.8	44.8	55.2
Age in years:								
Median	26	34	34	39	24	32	31	44
Interquartile Range	(5-50)	(5-47)	(26-52)	(33-70)	(8-45)	(10-43)	(14-49)	(25-53)

Table 1: Overview of all enrolled subjects. In total 129 GD patients, 13 GD carriers, 261 patients suffering from other LSDs and 148 controls were enrolled. The gender was not distributed equally among all four sub-cohorts, 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).

	Chitotriosidase	CCL18 Park	Glucosylsphingosine
	(n=233)	(n=207)	(n=521)
Cut point	>145	>166	>12
Sensitivity	91.7%	76.2%	100.0%
Specificity	86.1%	79.4%	100.0%
AUC and 95%CI in ROC Analysis	0.94 (0.89-0.98)	0.87 (0.80-0.93)	1.00 (1.00-1.00)

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

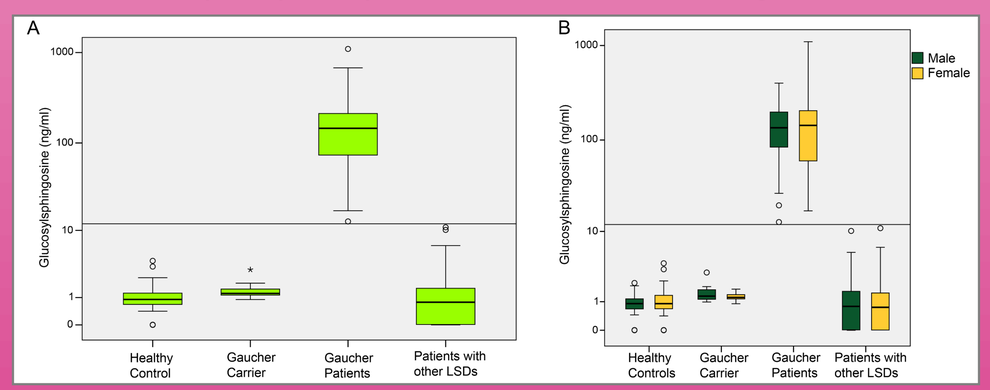

Figure 1: Glucosylsphingosine levels in the four sub-cohorts. Level of Glucosylsphingosine is illustrated in the entire cohort (A) and separated according to gender (B). Glucosylsphingosine in GD patients was compared to healthy controls, GD carriers and patients with other LSDs.

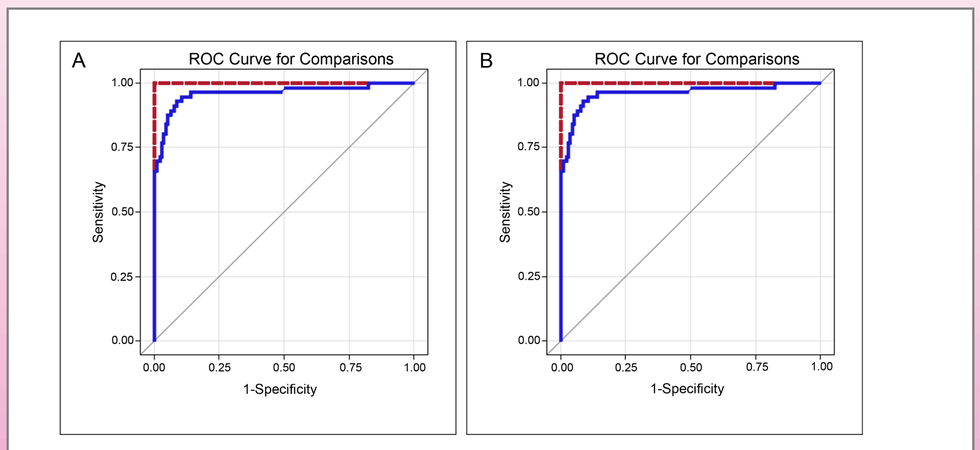

Figure 2: ROC curve analysis for comparison of Glucosylsphingosine with Chitotriosidase and CCL18/PARC. Glucosylsphingosine (A; red line; area under the curve (AUC) = 1.00) and Chitotriosidase (A; blue line; AUC = 0.96) as well as Glucosylsphingosine (B; red line; AUC = 1.00) and CCL18 (B; blue line; 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).

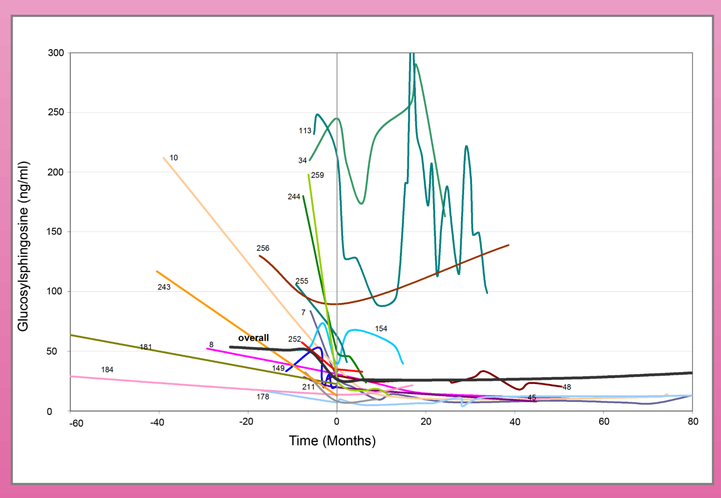

Figure 3: Monitoring of ERT by Glucosylsphingosine. The course of the Glucosylsphingosine was determined after onset of treatment, the time point zero defined as the first value after onset of therapy. The course for 19 GD patients undergoing ERT is shown.

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

Methods

Patients and blood samples:
Blood samples were obtained from patients enrolled by the Albrecht-Kossel-Institute for Neuroregeneration (AKos), informed consent was obtained from all probands. 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 (Niemann-Pick Type C disease, Fabry disease, Pompe disease, Krabbe 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 (in EtOH). After centrifugation at 4000 rpm for 2 minutes the clear supernatant was transferred into auto-sampler vials and injected into the HPLC-MS/MS system. Mobile phase used for gradient elution was 50 mM formic acid in water and 50 mM formic acid in acetonitrile/acetone (1/1, v/v). HPLC flow was set at 0.9 mL/min on an ACE 3 C8 column (50 x 2.1 mm) at 60°C, injection volume used was 5 µL. Retention time for the analyte was approximately 3.4 minutes and for the internal standard (lyso-Gb2, sufficient amount added if needed during sample preparation) approximately 3.6 minutes. For determination of free Glucosylsphingosine in plasma the API 4000 MS/MS system was used for electrospray ionization in MRM mode in positive mode at 500°C. For details please see Rolfs et al. 2013 [3].

Statistics:
Data was aggragated according to the ealiest measured value for GD patients before therapy and the highest biomarker value for controls if more than one measurement was available. This resulted in a sample of 148 healthy controls, 13 GD carriers, 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 to 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 (Glucosylsphingosie vs. chitotriosidase, Glucosylsphingosine vs. CCL18/PARC) paired sample statistical techniques were used [6]. The ROC curves were calculated using PASW Statistics 18, Release Version 18.0.2 (© SPSS, Inc., 2009, 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 analysed non-aggregated data for patients for whom several blood sample 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.

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