Quantification of glucosylsphingosine (lyso-Gb1) for the diagnosis and monitoring of Gaucher disease

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Introduction: Gaucher disease (GD) is an autosomal recessive disease caused and characterized by a deficient beta-glucocerebrosidase activity, resulting in accumulation of glycolipids in cells and tissues of the affected patients. Standard diagnostic procedures include measurement of enzyme activity, genetic testing as well as analysis of biomarkers. We present here the characteristics of the lyso-Gb1 (glucosylsphingosine) determination in clinical set-up as a clinically relevant and important alternative to less specific biomarkers, such as chitotriosidase and CCL18/PARC.

Materials and methods: The method is based on the extraction of biomarker lyso-Gb1 from dried blood spots (DBS) or plasma and specific determination and quantification by multiple reaction monitoring mass spectrometry (MRM-MS). The method validation process revealed the following characteristics for lyso-Gb1 determination: (i.) intra- and inter-assay precision with CV% < 10% for both DBS and plasma for all tested concentrations; (ii.) for accuracy CV% < 8% and excellent linearity for the analytical range; (iii.) reference values were determined on normal controls (average +2*STD) at <1.2 ng/mL (plasma) and <4.8 ng/mL (DBS); (iv.) pathological range 23.2 to 226.0 ng/mL (plasma), and 15.4 to 2836.0 ng/mL (DBS); (v.) LOD and LOQ was determined on blanks, (vi.) specificity and sensitivity 100 %; (vii.) lyso-Gb1 concentration is stable for at least 11 years in DBS stored at room temperature; (viii.) excellent correlation between plasma concentration and DBS.

Summary: Lyso-Gb1 was demonstrated to have the highest sensitivity and specificity to date for diagnosis and monitoring of Gaucher Disease.

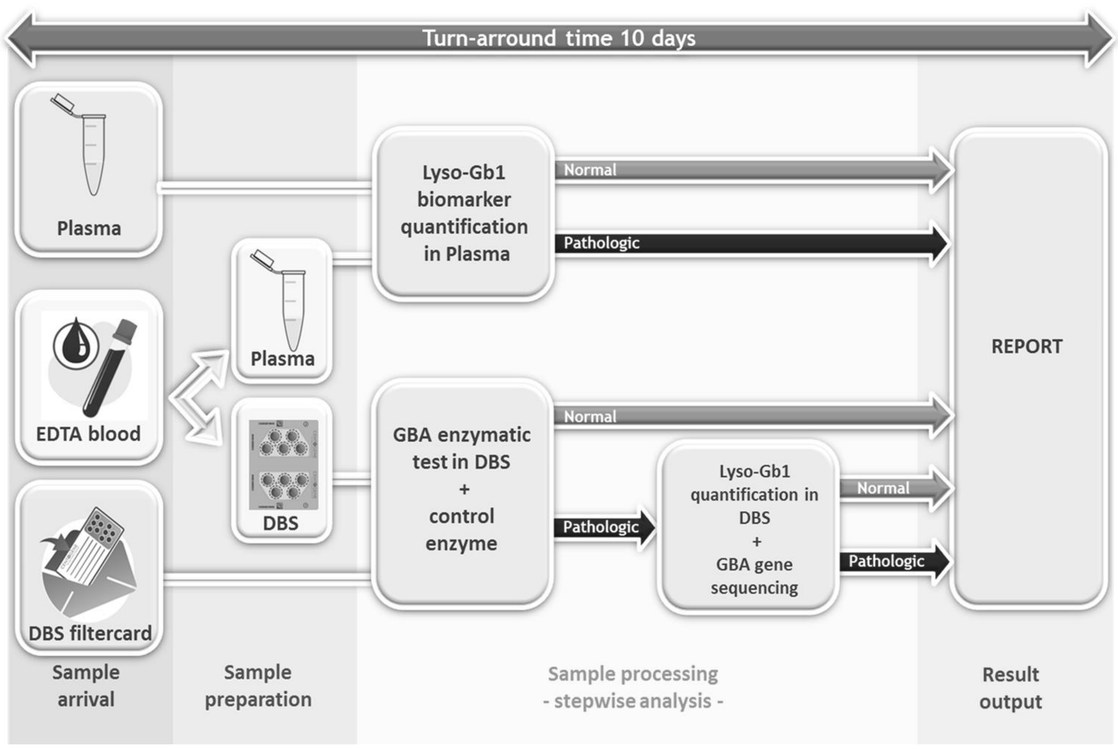

Figure 1: *Diagnostic flowchart for GD diagnostic*

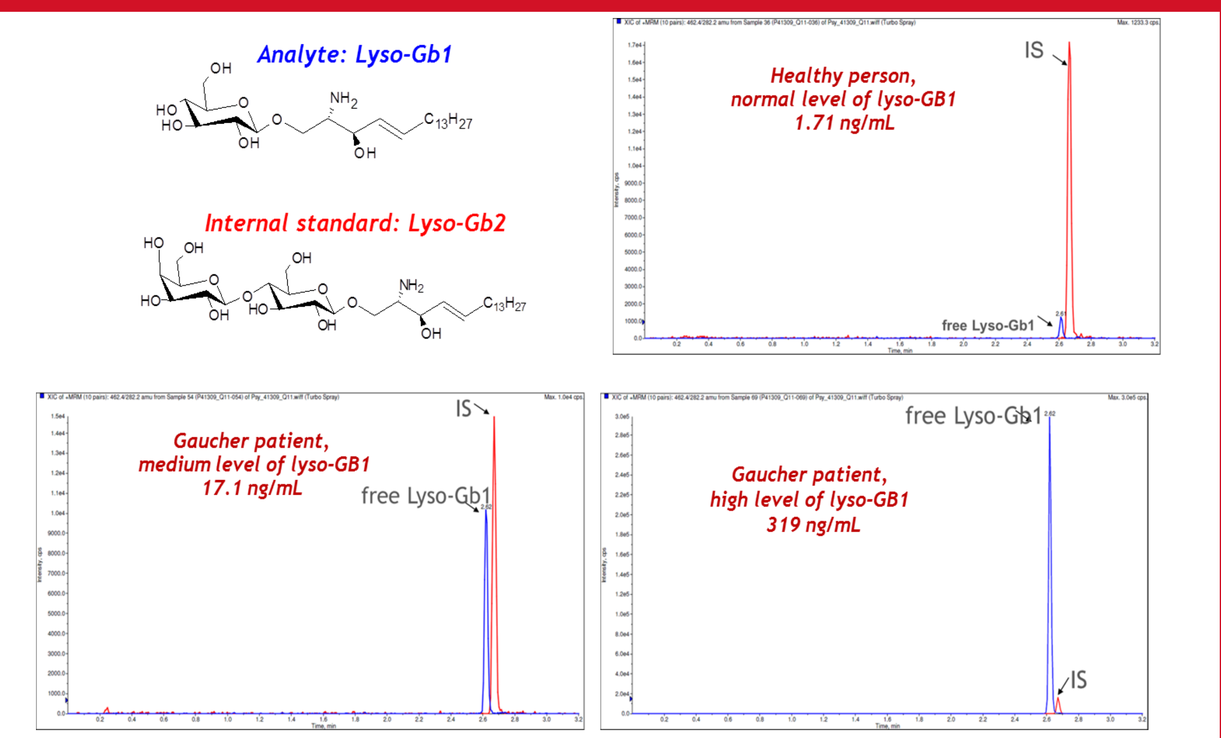

Figure 2: *Glucosylsphingosine (Lyso-Gb1) quantification by multiple reaction monitoring mass spectrometry (MRM-MS)*

Determination of lyso-Gb1 in DBS Validation of method		Result
Precision	Intra-assay	CV = 3.0 -8.3 %
	Inter-assay	CV = 2.5-5.3%
Accuracy	Intra-assay	CV = 0.4-5.6 %
	Inter-assay	CV = 0.4-7.6%
Specificity of the measurment		Through LC/ MRM-MS transition 462.3/282.2 and RT
Linearity of the measurments		0-2000 ng/mL
Normal range (N=50)		1.7-4.9 ng/mL
Reference value (mean+2STD)		< 4.8 ng/mL
Pathological range (N=359)		15.4-2836 ng/mL
Sensitivity		100%
Specificity of the method - GD patients vs. normal controls (using calculated 4.8 ng/mL reference value)		99.89%
Specificity of the method -lyso-Gb1 levels in other LSD patients (N=82)		99.82%
Limit of detection		0,48 ng/mL
Limit of quantification		0,97 ng/mL
Robustness (parameter – time from sample prep to measurement)		Lyso-Gb1 can be quantified up to 24h after extraction from plasma/DBS
Viability of DBS samples		Lyso-Gb1 could be measured in up to 11 years old cards
Short term stability up to 1 year (DBS stored at RT containing different concentrations of lyso-Gb1)		CV<5.1 %
Long term stability		10 years stability – study ongoing
Plasma –DBS conversion		Linear, conversion factor of 3.445 for non-hemolytic plasma

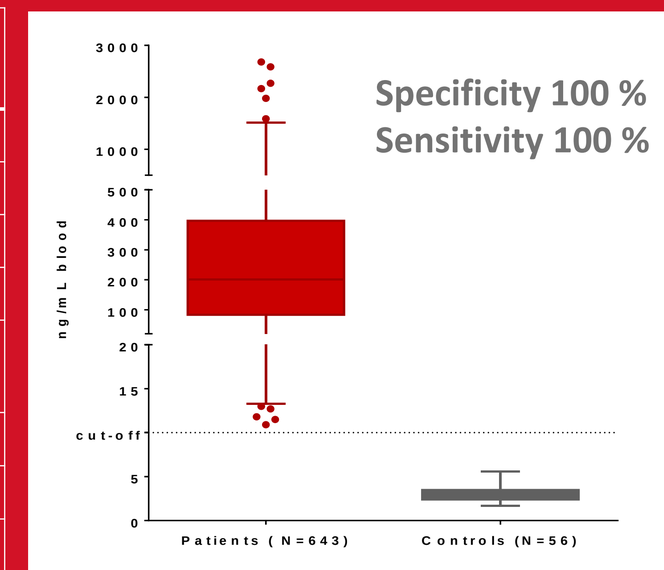

Figure 3: *Lyso-Gb1 has sensitivity of 100% and specificity of 99.89%*

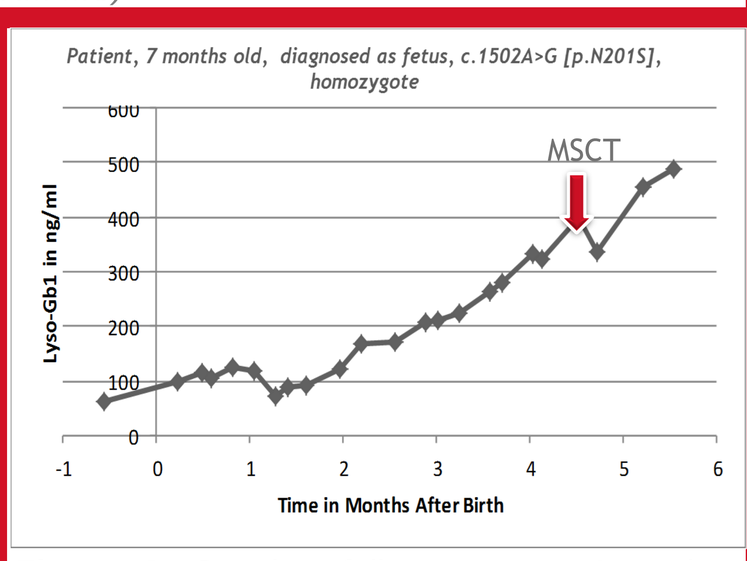

Figure 4: *Gaucher follow-up case 1: patient monitored every two weeks for 7 months; MSCT = Mesenchymal stem-cell transplantation**

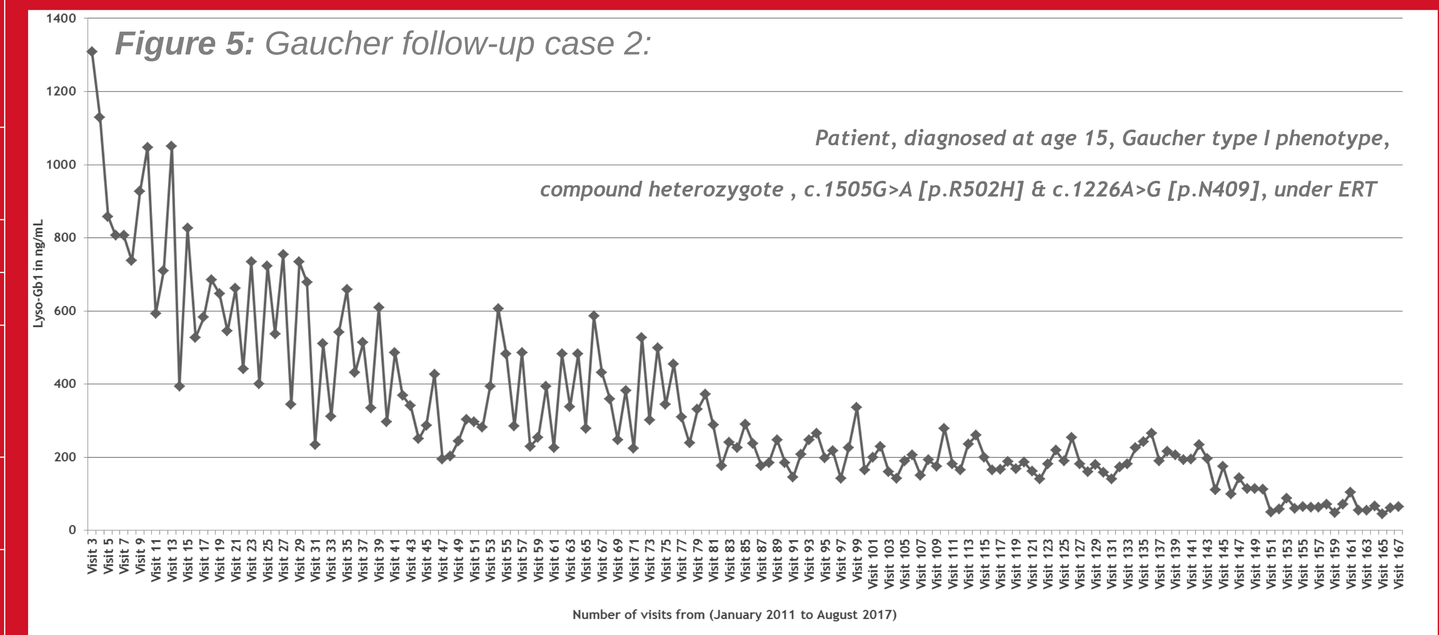

Figure 5: *Gaucher follow-up case 2:*

References

www.centomd.com
Rolfs et al,2013
CENTOGENE AG, US Patent 20140187439
* Collaboration with Karolinska Institute/Stockholm

Disclosure of conflict of interest:

This study was sustained in part by CENTOGENE AG, Rostock, Author of the presentation, Claudia Cozma, and 2 co-authors are employees of CENTOGENE AG, Rostock, Germany