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Glucosylsphingosine is a reliable response biomarker in Gaucher disease

To the Editor:

Gaucher disease (GD) is an autosomal recessive lysosomal disorder caused by impaired activity of β -glucocerebrosidase due to mutations in the *GBA1* gene. While glucosylsphingosine (Lyso-Gb1), the deacylated form of glucosylceramide, is an optimal diagnostic biomarker of GD,^{1,2} there is a substantial lack of studies identifying a valid biomarker

which quantifies patient response to therapy (rate or response biomarker). Difficulties in identifying rate biomarkers in patients with GD are due to its diverse natural history and wide variability in patient response to therapy.

Since any disease parameter that changes with therapy potentially can serve as rate biomarker, we assessed the value of key disease parameters such as biomarkers by retrospectively analyzed data that were obtained from a homogenous population of patients. This cohort of GD patients, all non-splenectomized GD, homozygote to the non-neuronopathic N370S (c.1226A>G) mutation in the *GBA1* gene and who received a low dose of enzyme replacement therapy (ERT, 15 units per kg per month), partially enabled us to overcome response variability and to assess the value of potential rate biomarkers more reliably. This study was approved by the ethics committee of Shaare Zedek Medical Center (0165-17-SZMC). See Supporting Information for detailed methods.

We longitudinally assessed the effect of ERT on key disease parameters in 25 GD patients (Supporting Information Table). Parameters included Lyso-Gb1 levels, platelet counts, hemoglobin values, and the normalized volumes of the spleen and liver (multiples of norms). Seventeen patients received an ERT of velaglucerase alfa (VPRIV®, Shire), four received imiglucerase (Cerezyme®, Genzyme), and an additional four received taliglucerase alfa (Elelyso®, Pfizer). One patient (female, treated with imiglucerase) was excluded from liver and spleen volume analyses as her pre-treatment measures were lacking.

Following ERT, Lyso-Gb1 levels decreased (25 patients; average: 4.6 measurements per patient; range: 2–8 measurements; median follow-up 46 months; range of follow-up 5–72 months, Supporting Information Figure 1), whereas platelet counts and hemoglobin increased (25 patients; average: 4.7 measurements per patient; range: 2–8 measurements; median follow-up 46 months; range of follow-up 5–72 months). Spleen volume clearly decreased while the change in liver volume was less clear (24 patients; average: 4.2 measurements per patient; range: 2–7 measurements; median follow-up 45.5 months; range of follow-up 6–72 months).

For each patient, disease parameters were normalized to pre-ERT values. For each disease parameter, all normalized measurements were pooled over the entire patient cohort and plotted by time since ERT introduction. Finally, a linear and exponential curve was fitted to the pooled data of each disease parameter. This analysis shows that the decay in Lyso-Gb1 levels following ERT therapy is well described by an exponential curve ($R^2 = 0.84$, Figure 1). The linear model provides a lower R^2 (0.68) and therefore is less accurate in describing the data. For all other disease parameters, fitting was far from optimal, both for the linear model (for changes in platelet count, hemoglobin, spleen volume, and liver volume $R^2 = 0.38$, 0.2, 0.35, and 0.01 respectively) and for the exponential model ($R^2 = 0.37$, 0.2, 0.36, and 0.01, respectively).

Using the derived equation for post-ERT exponential decay in levels of Lyso-Gb1 and in values of the multiple of norms for the spleen we determine the half-life for normalization of these two variables. Plasma levels of Lyso-Gb1 are half-normalized after 15.4 months (95% interval: 13.8–17.4 months) from the time of starting homozygous N370S GD patients on a low-dose ERT. The volume of the spleen is

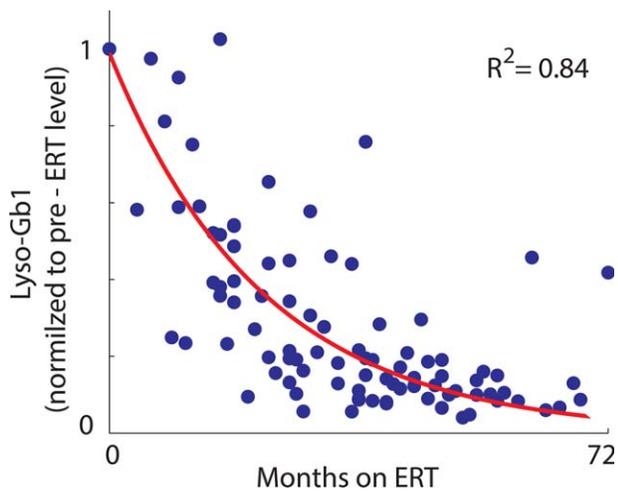


FIGURE 1 Exponential decay in Lyso-Gb1 plasma levels following ERT. Lyso-Gb1 levels were normalized based on the pre-ERT level for each patient and an exponential curve (red line) was fitted to the pooled data.

half-normalized at a much later point in time, after 122 months (95% interval: 97–167 months).

We also assessed the feasibility of using the calculated half-time of decay for Lyso-Gb1 as a biomarker for spleen normalization with different ERTs. For this purpose, we analyzed data from patients who received velaglucerase alfa (17 patients) separately from data of those who received imiglucerase or taliglucerase alfa (7 patients). The calculated half-life of Lyso-Gb1 in the velaglucerase alfa group was 14.8 months (95% interval: 13.2–16.9 months) and for the imiglucerase/taliglucerase alfa group was 17.6 months (95% interval: 13.9–24.1 months). Analysis of spleen volume normalization showed that it followed the same pattern. In patients on velaglucerase alfa the calculated decay half-life for spleen shrinking was 118 months (95% interval: 90–172 months), notably shorter than the value calculated for patients receiving imiglucerase/taliglucerase alfa treatments (138 months, 95% interval of 92–275 months).

Elevated Lyso-Gb1 levels in GD patients have been previously reported,^{1,2} and was shown to be superior diagnostic biomarker for the disease when compared to chitotriosidase and CCL18.^{1,2} This lipid is considered a contributing factor to the pathophysiology of the disease.^{3–5} The current retrospective study demonstrates that Lyso-Gb1 is also valuable as a rate biomarker (type I or pharmacological biomarker⁶). Rate biomarkers allow monitoring patient response to ERT, predicting treatment outcome and comparing efficacy among different ERTs and between ERTs and other therapeutic modalities.

Decrease in Lyso-Gb1 levels following ERT in GD patients was shown in previous studies.^{1,2} However, these studies included patients with wide variation in GBA1 mutation types, patients with and without spleen, different modes of therapy (ERT and substance reduction therapy), and variable dose of ERT. In addition, these studies did not measure the utility of Lyso-Gb1 as a rate biomarker and did not compare it to other disease parameters which are commonly employed as such. Hence it has remained difficult to demonstrate the precise pattern of decay of this substance. Our study addresses this caveat.

We conclude that Lyso-Gb1, which is presently the most specific and sensitive diagnostic biomarker of GD, is also the most reliable rate biomarker. In addition it has the potential to promote resolution of persisting issues in the treatment of GD such as the optimal dose of ERT, the need to shorten the duration of clinical trials, and predicting long-term organ response to therapy.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

DA: Analysis and interpretation of data and manuscript preparation. TD: Acquisition of data and critical revision of the manuscript. MBC: Acquisition of data and critical revision of the manuscript. CC: Acquisition of data, interpretation of data, and contribution to the manuscript preparation, critical revision of the manuscript. MH: Contribution to the manuscript preparation, critical revision of the manuscript. SE: Contribution to the manuscript. AR: Contribution to study conception and design, contribution to the manuscript preparation, critical revision of the manuscript. AZ: Contribution to study conception and design, contribution to the manuscript preparation, critical revision of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Heterogeneous hemoglobin lower thresholds in clinical laboratories

To the Editor:

Accurate and harmonized definitions of anemia are critical for clinical management of patients and for ascertaining the epidemiology of the problem in public health. We surveyed laboratories participating in the UK External Quality Assurance programme (The UK NEQAS, a charitable consortium of external quality assessment laboratories), to determine the thresholds they used to define anemia. We found that while most laboratories provide sex-specific thresholds, few laboratories used distinct thresholds in pregnancy, children, postmenopausal women, or elderly people. Lower thresholds (often used clinically to define anemia) varied markedly between laboratories, especially among women, children and in pregnancy. Most laboratories cited textbook

sources for their thresholds; the thresholds used were often discordant with that cited in the reference. The evidence underlying hemoglobin thresholds to define anemia remains limited, thus heterogeneity between laboratories is perhaps not unexpected.

Anemia is considered to exist when the hemoglobin concentration is inadequate for physiologic oxygen carrying needs. Accurate definition of anemia is critical for clinical practice, understanding anemia epidemiology, and planning public health interventions. However, there is uncertainty about the hemoglobin thresholds at which anemia should be defined. For example, in adult males, the World Health Organization (WHO) defines anemia as hemoglobin <130 g/L,¹ whereas the United States (US) Centers for Disease Control and Prevention (CDC) defines anemia as hemoglobin <135 g/L.² In pregnancy, CDC varies the definition of anemia by gestational age (CDC 2011), whereas WHO maintains <110 g/L throughout (WHO 2011). Furthermore, prominent hematology textbooks cite a variety of thresholds. Using WHO definitions, anemia affects 41.7% of children and 32.8% of women worldwide (predominantly, but not exclusively, in low income countries).³

We assessed the degree of harmonization of anemia definitions used in clinical practice among the 808 laboratories registered with The UK NEQAS. NEQAS provides a comprehensive external quality assessment service to most laboratories supporting hospitals in the UK National Health Service (NHS) in addition to other international sites. Approval was granted by Monash University's Human Research Ethics Committee (CF16/1925–2016000981). Of the 600 clinical laboratories or diagnostic sites registered with NEQAS to which this survey was relevant and circulated, 208 laboratories (35% overall, 60% for UK respondents) across 14 countries responded, predominantly from Europe (199/208, 95.7%), including two-thirds UK (140/208, 67.3%) (Supporting Information Table S1). Questionnaires from NEQAS are completed by the laboratory manager, similar senior scientist, or medical or scientific head of department.

More laboratories cited textbooks (82/208, 39.4%), predominantly Dacie and Lewis' *Practical Hematology* (52/208, 24.9%), than WHO guidelines (23/208, 11.1%). Twenty-three percent (48/208) reported calculating their own reference ranges (Figure 1A). Most laboratories provided sex-specific adult [male (187/208, 89.9%) and female (164/208, 78.8%)] hemoglobin thresholds to define anemia (Figure 1B). Figure 1C shows thresholds used. The most common threshold in adult males was <130 g/L (126/187 laboratories, 67.7%, range 120–140 g/L). For females, 21% of laboratories (44/208) used the same threshold as for males; among those using separate thresholds, half used <115 g/L (81/164, 49.4%, range 90–125 g/L). Only 8% of laboratories (16/208) used separate thresholds for pregnancy (range 90–120 g/L), and 4% (8/208) for premenopausal and postmenopausal females. Sixty-one percent of laboratories (127/208) used specific thresholds for children. The most common threshold was <110 g/L in young children (1–5 years, 40/127 laboratories, 31.5%, range 90–130 g/L) and <115 g/L in older children (5–13 years, 80/127, 63.0%, range 90–130 g/L). Only 10% of laboratories (21/208) provided distinct thresholds in the elderly, with just one providing sex-specific (>65 years) thresholds. No laboratory varied thresholds by ethnicity.