Importance of lyso-GL-3 (lyso-Gb3) for primary diagnostics of Fabry disease: two-year experience in a daily routine laboratory

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[1] Introduction:

Diagnostics of Fabry disease (FD) is challenging particularly for the identification in women suspicious to disease in a daily clinical laboratory. Currently used mass spectrometry-based diagnostic assays may detect a normal alpha-galactosidase activity in female Fabry patients thus women at risk might be missed and consequently undiagnosed (1). De-acylated GL-3 or globotriaosylsphingosine (lyso-GL-3, lyso-Gb3) was described as a potential biomarker in Fabry disease (2, 3).

We introduced a standardized fully validated and certified in vitro diagnostic (IVD) lyso-GL-3 assay for the use in a daily clinical laboratory. This assay includes a seven-point quality calibrator as well as a three-point quality control material for the quantitation of lyso-GL-3 in Dried Blood Spots (4).

In our routine laboratory, alpha-galactosidase activity measurement and in parallel the quantitation of lyso-GL-3 in more than 5,000 Dried Blood Samples derived from woman suspicious to Fabry disease were performed. Samples positive for enzyme and/or lyso-GL-3 levels were finally genetically analyzed. This international diagnostic service is supported by Sanofi Genzyme.

[2] Challenges:

Fabry disease is identified in male by determination of alpha-galactosidase activity and positive cases are confirmed by genetic testing (Figure 1).

**Fabry diagnostics in male:**

- **alpha-galactosidase activity testing**
  - **Negative test result**
  - **Positive test results**
  - **No further action required**
  - **Genetic confirmatory testing**

Optional: genetic and/or lyso-GL-3 testing in patients with family history or inconclusive GLA enzyme test.

Figure 1 Illustration of the diagnostic procedure for the identification of Fabry disease in men.

[3] Fabry diagnostics in females:

- **alpha-galactosidase activity AND lyso-GL-3 testing**

  1. **Negative GLA, Negative lyso-GL-3**
     - Classical FD very unlikely.
     - Genetic confirmatory testing!
  2. **Negative GLA, Positive lyso-GL-3**
     - FD very likely.
     - Genetic confirmatory testing!
  3. **Positive GLA, Negative lyso-GL-3**
     - Classical FD very unlikely.
     - Genetic confirmatory testing!
  4. **Positive GLA, Positive lyso-GL-3**
     - Classical FD very likely.
     - Genetic confirmatory testing!

**CAVE: Genetic testing recommended in all women with family history or specific clinical symptoms!**

Scenario 2: less female FD’s are missed!

Figure 2 Illustration of the improved diagnosis procedure for the identification of Fabry disease in women.

[4] Conclusion:

A primary genetic screening of a large cohort of females (also irrespectively of the indication of Fabry disease) would result in the detection of genetic variances of unknown significance (VUS). This generates an uncertain situation for women and might lead to an over-diagnosis (and treatment) without any further differential diagnosis. Alternatively, alpha-galactosidase and simultaneous lyso-GL-3 testing has the benefit of detecting a significant higher number of females with Fabry disease. Our data show the importance of the quantitation of lyso-GL-3 using a validated mass spectrometry assay not to miss any woman at risk to start therapy adequately. To minimize the number of potential false-negative cases, genetic testing is always mandatory in all females with family history, children as well as in all females with specific clinical symptoms.

References:

1) Mehta A. et al. 2006
2) Aerts J.M. et al. 2008
4) Nowak A. et al. 2017