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 detected 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**).

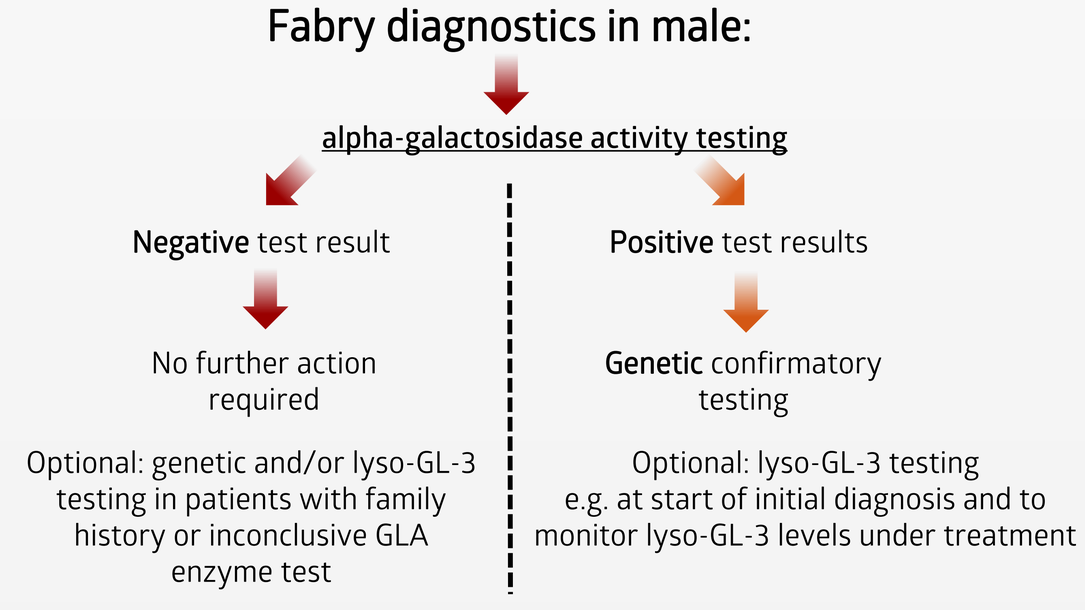

Figure 1 Illustration of the diagnostic procedure for the identification of Fabry disease in men.

Might miss up to 20% of females with Fabry disease if lyso-GL-3 testing IS NOT performed!

[3] Fabry diagnostics in females:

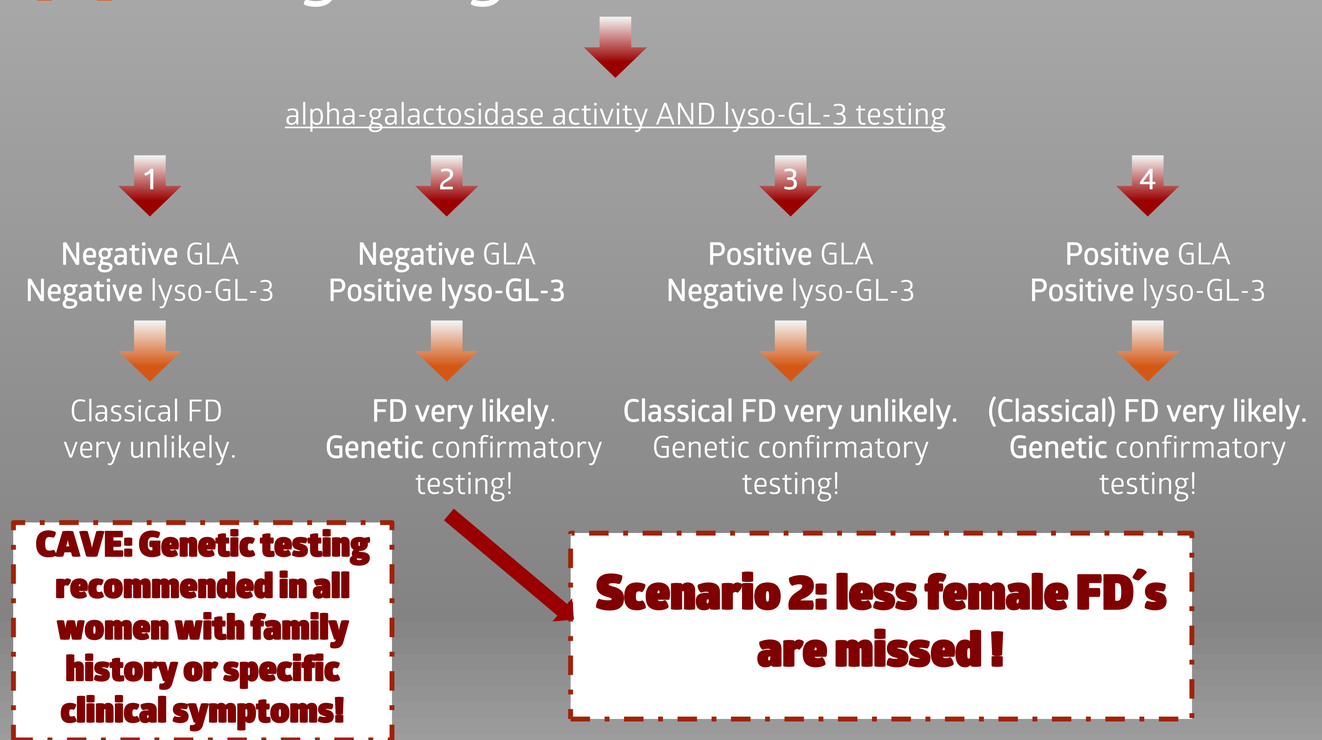

Figure 2 Illustration of the improved diagnosis procedure for the identification of Fabry disease in women.

To improve the sensitivity and minimize false-negative results (approx. 20%) by using alpha-galactosidase activity and lyso-GL-3 testing in women, a new algorithm was established and presented in Figure 2.
Approximately 5,000 samples from women suspicious to Fabry disease were tested for alpha-galactosidase enzyme activity AND lyso-GL-3:

- >50 enzyme positive women PLUS positive genetic tests.
- Additional > 20 enzyme negative women PLUS positive lyso-GL-3 test were identified with final positive genetic test (potential patients from known families were excluded).

[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.

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References:
1) Mehta A. *et al*. 2006
2) Aerts J.M. *et al.* 2008
3) Smid B.E. *et al*. 2016
4) Nowak A. *et al*. 2017