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GL-3: a urinary biomarker in Fabry disease?

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At the Forefront of Therapies for Rare Diseases™

Fabry disease

Slide 3

- Fabry disease (FD) is an X-linked lysosomal storage disorder characterized by deficiency of α -galactosidase A (α -Gal A).
- Accumulation of glycolipid isoforms of globotriaosylceramide (GL-3) with progressive, multi-organ disease.
- Large variability of clinical phenotypes and clinical progression (chronic, sub-chronic, acute).
 - Cutaneous
 - Peripheral nerves
 - Cardiac
 - Renal
 - Brain
 - Males and females affected
- GL-3 can be measured in body fluids (plasma, urine), in homogenized tissue, or estimated on histology (stained inclusions).
- Enzyme replacement therapy available (agalsidase beta in US; beta & alfa ex-US).

Marker of Fabry disease : US landscape

Slide 4

- GL-3 is a relevant biochemical marker: substrate for α -Gal A, pathologic accumulation in tissue.
- GL-3 is not a surrogate marker: no satisfactory demonstration of correlation with clinical disease progression/improvement.
- GL-3 was primary outcome for pivotal phase 3 study which led to conditional approval of agalsidase beta (2003).
- Clinically relevant primary outcome have not been successful (pain, composite of clinical events).
- GL-3 to be evaluated in 'peri-tubular capillary cells of the kidney' (PTCs) and 'clearance' was the requested outcome.
- GL-3 semi-quantitative histology reading or quantitative assays not standardized.
- GL-3 in urine not evaluated as a primary outcome.

GL-3 evaluation in peri-tubular capillaries (PTCs)

Slide 5

- PTCs are histologically convenient (number and identification) and 'clearance' possible (cell renewal within months).
- PTCs are not part of the glomerulus.
- PTCs GL-3 accumulation not always predominant kidney pathophysiology. Growing body of knowledge points to the podocyte (and tubular cells) as the central player.
- Growing body of knowledge indicates semi-quantitative histology could be subjective and not sensitive enough .
- Fabry patients can have severe signs and symptoms and minimal PTCs GL-3 accumulation.
- Patients can have proteinuria, isosthenuria, or decreased renal function but minimal PTCs GL-3 accumulation.
- On the same slide, PTCs can have minimal GL-3 accumulation, while podocytes or tubular cells are overloaded with GL-3.
- On the same slide, PTCs can be cleared with ERT but podocytes, generally, are not.
- Kidney biopsy(ies) not always acceptable to patients, IRBs, and/or physicians.

Is there another way to evaluate GL-3?

Slide 6

- Can the quantitative measurement of GL-3 in urine replace semi-quantitative histologic evaluation in PTCs?
- Same marker, same organ but ...

Current situation with urinary GL-3

Slide 7

Weaknesses

- Generally not available to clinicians.
- Not a surrogate marker .
- Essentially, a marker of kidney disease but not all Fabry patients have kidney problems.
- No GLP assay.
- Variability of methods (research tool).
- Mishandling of sampling (sonication) and timing (spot versus 24 hour).
- Variability in expression of results (multiple normalizations, no normalization).
- Confusing literature.

Strengths

- Directly linked to disease pathophysiology.
- Produced by desquamation of tubular cells, podocyturia & plasma filtration.
- If plasma component negligible, exclusive renal origin.
- Quantifiable and objective.
- Practical to evaluate treatment effect.
- Non traumatic.

We can use these strengths and address many of these weaknesses

Amicus developed a urinary GL-3 GLP assay¹

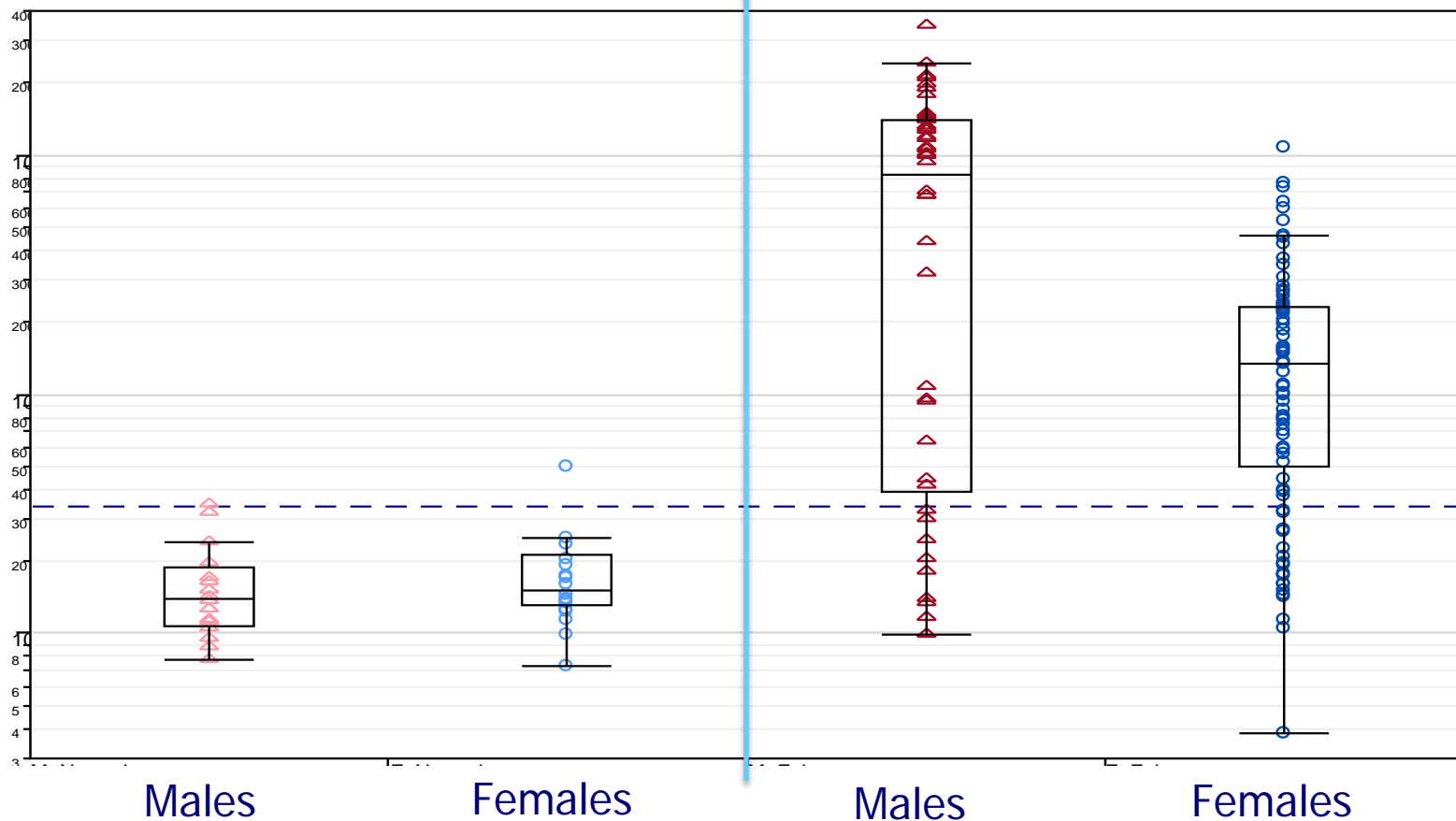
Slide 8

- First fully validated GLP assay to measure urine GL-3 (LC-MS/MS).
- Can accurately quantify C22:0 and C24:0 isoforms down to an LLOQ of 1 ng/mL.
 - Predominant isoforms in kidney cells
 - Not predominant isoforms in plasma
- Validation parameters established as per 2001 US FDA Bioanalytical Method Validation guidance for industry.
- Validated assay accurately measure levels of C22:0 and C24:0 isoforms in 38 normal urine samples.
- Assay helps define upper limit of normal in healthy individuals (33.8 ng/mg cr) for urine GL-3 as measured by a combination of C22:0 and C24:0.

¹Sitaraman S, Meyer M, Schiffmann R. First fully analytically validated GLP assay to measure kidney specific isoforms of GL-3 in urine in Fabry disease. Presented at SSIEM congress. Geneva, September 2011

Urinary GL-3 in normal subjects and Fabry patients (ng/mg creatinine)

Slide 9

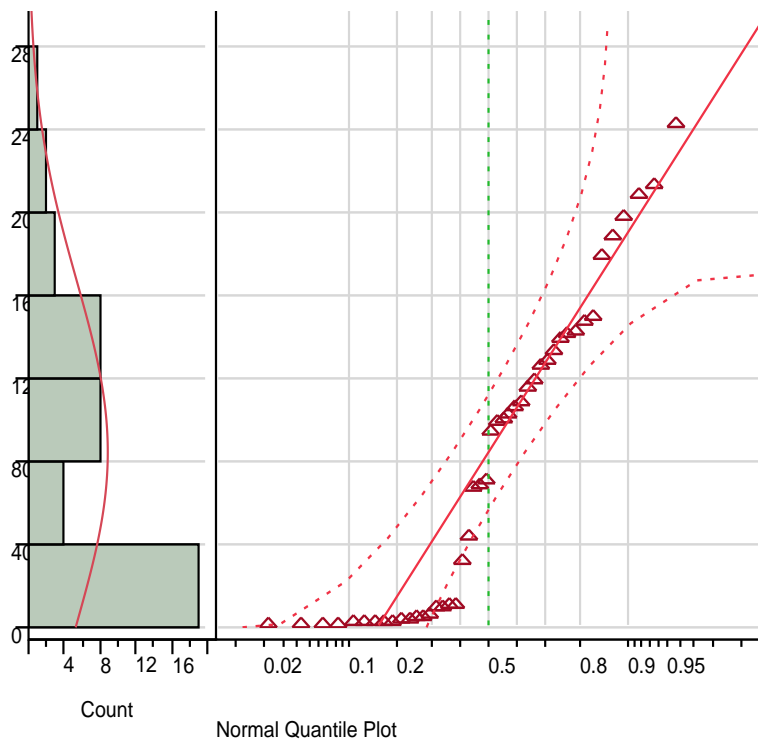


Normal

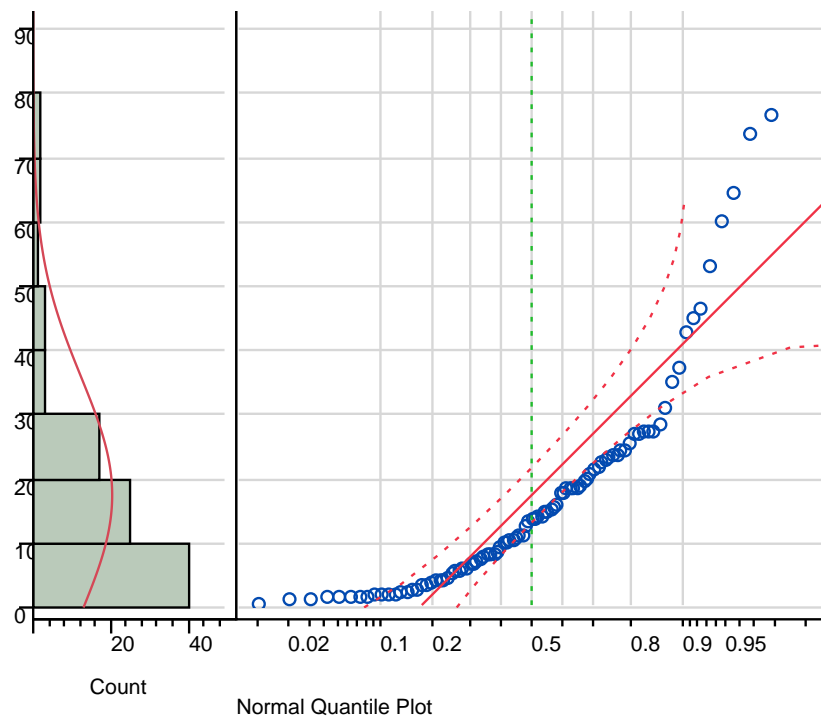
Fabry

Urinary GL-3 distribution in males and females with Fabry disease

Slide 10



Males



Females

Next steps urinary-GL3 assay

Slide 11

- Clinical validation 'as we go'.
 - Intra-patient variability over time.
 - Correlation with Fabry disease status and disease progression.
 - Response to treatment.
 - Others?
- Use for clinical practice / labeling.
 - Can u-GL3 help diagnosis or monitoring of Fabry patients?
 - Current ERT label: Agalsidase beta is indicated for use in patients with Fabry disease. Fabrazyme reduces (GL-3) deposition in capillary endothelium of the kidney and certain other cell types (see CLINICAL STUDIES).
- Access to urinary GL-3 assay for clinicians and patients.
 - On request?
 - Commercial?

Concluding remarks

Slide 12

- Because of small number of patients, especially in clinical studies, biomarkers are essential to study rare diseases.
- The establishment of clinical surrogacy requires many patients, long follow-up and 'simple' diseases.
 - From total cholesterol to LDL-cholesterol: 10^5 patients, 40 years.
 - HDL-cholesterol is still not an surrogate marker.
 - Hemoglobin A1c has been down graded.
 - HIV is an infectious disease (HIV RNA copies).
- Each Orphan disease is unique: Fabry \neq Gaucher \neq Pompe. However, some common general principles must apply.
- We need to work together.