DIAGNOSIS AND BIOCHEMICAL FOLLOW-UP OF FABRY DISEASE - INCLUDING CURRENT STATUS OF KIDNEY AND HEART BIOMARKERS

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Disclosure of Interests

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Overview

• Clinical case
• Diagnosis
• Biomarker aspirations
• Substrate-related biomarkers
• Pathophysiology-related biomarkers
  – TRAWLING AND ANGLING
• Clinical biomarkers
Case

- 42 year old man works in information technology.
- Chest pain which occurs occasionally lasting approximately 10 minutes and rarely palpitations.
- Routine health screen arranged through his employers.
- ECG abnormal
- Echocardiogram revealed concentric left ventricular hypertrophy with apical IVS of 23mm
- Hand pain in childhood until his teenage years.
  - now, if feeling febrile, unwell or exercising may have hand pain
- Abnormalities of sweating (anhidrosis) which exacerbate sensitivity to heat.
- Punctate red rash - always been present
  - bathing trunk region, periorally and periumbilically.
- Abdominal pain in his teenage years and now has occasional diarrhoea, sensation of abdominal fullness and nausea.
- Headaches associated with visual disturbance and memory loss in 1999
- Tinnitus, symptomatic postural hypertension and vertigo.
- High tone sensory neural hearing loss
- Fatigued and has less stamina than previously,
- Non reversible oedema and some calf pain.
**MEDICATIONS:**
- Solpadene for migraine

**FAMILY HISTORY:**
- He is unaware of anybody in the family with similar problems although his maternal cousin had renal failure.

**INVESTIGATIONS:**
- **Haematology:** n/a
- **Biochemistry:** Creatinine 70umol/l  E-GFR MDRD >90ml/min
  - EDTA GFR 84ml/min
  - Urine protein creatinine ratio 13mg/mmol
- **Lipids:**
  - Cholesterol 2.8mmol/l  Triglycerides 0.8mmol/l

**Pure tone audiogram:**
- Bilateral high tone sensory neural hearing loss worse on the left than the right.
Diagnostic investigations:
- Plasma alphagalactosidase A 1.1 (normal 8.9 – 39)
- Leucocyte alphagalactosidase 0.5 (normal 21.9 – 50.7)

Mutation analysis:
- N34D
- In addition there are polymorphisms c.370-81del5bp in intron 2 and c.640-16G>A in intron 4
Fabry: Clinical, Biochemical or Genetic definition?

Organisation of GLA Gene

5'UTR → Ex1 → Ex2 → Ex3 → Ex4 → Ex5 → Ex6 → 3'UTR

α-galactosidase A

KDIGO Controversies Conference on Fabry Disease | October 15-17, 2015 | Dublin, Ireland
a

**Laboratory Diagnostics in Fabry Disease: Males**

Clinical suspicion of Fabry disease

Measure α-galactosidase A activity in leukocytes (dried blood spot)

- 'Zero' activity: Fabry disease
  - Disease-causing mutation: Confirmation of Fabry disease
  - Decreased activity: Attenuated form of Fabry disease or Not Fabry disease
  - Normal activity: Not Fabry disease "Phenocopy"

b

**Laboratory Diagnostics in Fabry Disease: Females**

Clinical suspicion of Fabry disease

Measure α-galactosidase A activity in leukocytes (dried blood spot)

- Low activity: Normal activity
  - Sequence GLA gene
  - Disease-causing mutation: Confirmation of Fabry heterozygote
  - No disease-causing mutation: Individual evaluation
Known sequence variants

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Determining clinical significance of novel coding variants identified in the GLA gene
Chronic kidney disease and an uncertain diagnosis of Fabry disease: approach to a correct diagnosis

- Gold standard for FD nephropathy - characteristic storage on electron microscopy (EM) in a kidney biopsy in the absence of medication that may induce similar storage.

- Possible criteria to confirm FD nephropathy - 'renal cysts', 'Maltese cross sign', 'immunohistochemical staining of Gb3 in urine' and 'high urinary Gb3'; rejected

- Possible criteria to exclude FD nephropathy: 'absence of renal cysts', 'small kidneys' and 'high protein excretion' were rejected

- Urinary Gb3 may be increased in other kidney diseases

- The 'Maltese cross sign' and 'high urinary Gb3' were selected as red flags to suggest the possibility of FD nephropathy, not sufficient for a definite diagnosis of FD nephropathy.
Is there evidence that biomarkers help assess any of the following in Fabry Disease?

• Diagnostic or screening
• Phenotype definition
• Natural history
• Risk prediction
• Preclinical disease
• Clinical and biological heterogeneity
• Surrogate endpoint in clinical interventions
• Response to treatment
• Prognosis

‘Biomarker’

• Physiological
• Substrate derived-

– Beware the self fulfilling reduction of substrate and substrate derived ‘biomarkers’ by cognate enzyme

– This confirms enzyme activity and substrate accessibility but not clinical response
Substrate related biomarkers
Possible roles?

• Diagnosis
• Mutation pathogenicity
• Prognosis
• Disease status
• Response to therapy
• Clinical effects of antibodies
GB3 is the substrate for alpha galactosidase A
Relationship to mutation?

Urinary globotriaosylceramide excretion correlates with the genotype in children and adults with Fabry disease

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32 children and 78 adults
Significant relationship between urinary Gb3 and -mutation (missense, nonsense, frameshift, and splice-site defects): p=0.0007
Urinary Total Globotriaosylceramide and Isoforms to Identify Women With Fabry Disease: A Diagnostic Test Study

- 6 parameters
  - ratio of Gb3-24 to urinary AGAL activity;
  - Gb3-24;
  - ratio of Gb3-24 to Gb3-18;
  - Gb3-22;
  - Gb3-16;
  - total Gb3

- ‘highly informative for the diagnosis of Fabry disease independent of the presence or absence of CKD (area under ROC curve, 0.876-0.927; all P < 0.001).’
Relationship to therapeutic response?

Changes in plasma and urine globotriaosylceramide levels do not predict Fabry disease progression over 1 year of agalsidase alfa.

- Change from baseline eGFR predicted by
  - Baseline eGFR,
  - age at first dose,
  - baseline urine GB3 excretion,
  - baseline and change from baseline urine protein excretion
  - Change from baseline urine and plasma GB3 (baseline and change from baseline) concentrations did not predict change from baseline estimated glomerular filtration rate.

- No predictors of left-ventricular mass index were significant
Relationship to antibodies?

- Urinary GB3 increases in patients with neutralising antibodies

Fig. 6. Effect of IgG antibodies on the biological activity of agalsidase alpha. The filled symbols represent the patients who had demonstrated a persistently positive IgG antibody response while being treated and the open symbols represent patients who had no IgG antibody response (squares) or only a transiently positive response (triangles). The n indicate the number of patients in each category at baseline. For the IgG-negative group in the urine Gb₃ plot, baseline n is 10, reflecting the fact the patient who underwent a kidney transplant was not included in any renal analyses.

Schiffmann 2006; Vedder 2008
Adjusted estimated survivor functions (Cox proportional hazard model) for increasing urinary Gb3 values.
Lyso-Gb3

Gb3

lyso-Gb3

Fabry disease

LsyoGb3 levels

AGAL Activity
Fabry disease: Pedigree

Disease status and severity?

Variations in the GLA gene correlate with globotriaosylceramide and globotriaosylsphingosine analog levels in urine and plasma

- Individual profiles of Gb₃ and lyso-Gb₃ and analogs correlate with phenotypic data.
- Diagnostic tool to discern classical FD, cardiac variants and patients without FD.
- Lyso-Gb₃ analog at m/z 836 might be an earlier biomarker of progressive heart disease.
- Plasma and urine lyso-Gb₃ constitute clinically useful biomarkers of FD.
Pathophysiology-related biomarkers
Markers of pathophysiology

• ‘After the event?’
• Disease processes
• Translate into clinical practice?
• Potential for new interventions?
The identification of new biomarkers for identifying and monitoring kidney disease in pediatric Fabry and type-I diabetic patients

Schematic representation of a typical proteome of urine from pediatric Fabry disease patients prior to ERT. Proteins are represented as % fmol of protein of total proteins detected.

Urinary prosaposin concentrations pretreatment and post-treatment in typical and atypical responders. Error bars represent mean ± SD.
Typical UPLC–MS/MS chromatogram of the lower MW cut off fraction from patient urine showing the 10 min assay developed for the quantitation of individual Saposins A, B, C, D and GM2AP. For each peptide and internal standard, 2 transitions we used, one for quantitation and one for secondary confirmation purposes.
A distinct urinary biomarker pattern characteristic of female Fabry patients that mirrors response to enzyme replacement therapy.

Compiled urinary protein profiles of female Fabry patients (a) and healthy controls (b) included in the training cohort.

ROC curves for differentiation of Fabry female patients and female healthy controls in the training set upon complete take-one-out crossvalidation (a) and in the independent validation set (b).

Markers of fibrosis

- MMP-9 TIMP-1
- TIMP-2
- MMP-9 significantly higher in AFD
- Positive correlation with MSSI
- Negative correlation endocardial FS

Shah et al 2007 JIMD 30:88

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Fabry disease
Cardiovascular involvement

- Elevated blood biomarkers for fibrosis
  - In patients ± fibrosis
  - Not helpful for either characterizing cardiomyopathy or staging the disease*

*Perhaps because of other organ involvement, e.g., kidneys; reduced eGFR may play a role in collagen marker clearance
NT-pro BNP

- Correlation with
- Age
- Cr
- Left atrial index
- E/Ea
- Abnormal ECG
Cystatin C:
- good detection early renal disease,
- strong correlation with advanced renal disease and MSS1,
- weak correlation with cardiovascular, ocular, CNS
- ?ERT effect
Clinical biomarkers
Clinical biomarkers
Histological examination of cardiomyopathy pts with Chinese later onset mutation

IVS4+919>A
Endo myocardial biopsies
22 patients 17 ERT
5 no ERT
-GB3 in cardiomyocytes pts ERT <3 years
-no inclusions in capillary endothelial cells

Serum Lyso-GB3 increased after 11 months even when LVMI decreased
Agalsidase benefits renal histology in young patients with Fabry disease

Segmental podocyte foot process effacement, female, 15 years, patient 5 (re-biopsy after three years treatment with agalsidase alpha 0.2 mg/kg/eow).

Figure 3.

Baseline biopsy specimen (upper panel) shows full score of GL3 deposits. Re-biopsy after 5 years of ERT, 1 mg/kg every other week (lower panel), shows almost complete clearance of deposits in a 7-year-old boy (patient 1). Shown are light microscopic images of hematoxylin and eosin sections (A), PAS sections (B), and osmicated toluidine sernithin sections (C) and electron microscopic image (D). Original magnification: × 1000 in A; × 1000 in B; × 400 in C; × 2000 at baseline and × 1500 at 5 years in D.

Nephron. 2015;129(1):16-21
LVH neg: Mean septal T1 lower limit of normal; function abnormalities
TDI in FD cardiomyopathy

Early Detection of Fabry Cardiomyopathy by Tissue Doppler Imaging
Maurizio Pieroni, Cristina Chimenti, Roberta Ricci, Patrizio Sale, Matteo Antonio Russo and Andrea Frustaci

Fabry disease
Cardiovascular outcomes
Classic vs Cardiac variant

• Primary endpoint: Composite:
  - New onset AF, NYHA ≥ 3 symptoms, device insertion for bradycardia & cardiac death
• Incidence: 2.64 per 100 person-years (CI 1.78 to 3.77).

• Significant independent predictors:
  - Mainz Severity Score Index score (HR 1.05, CI 1.01-1.09, p=0.012)
  - QRS duration (HR 1.03, CI 1.00-1.05, p=0.020)
  - NOT genotype
Conclusions

• Diagnosis
  – Genetic, biochemical, substrate, clinical

• Biomarkers
  – Substrate
  – Pathophysiology
  – Clinical

• Nil universally satisfactory for diagnosis, prediction or monitoring