Biomarkers of Fabry Disease Nephropathy

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It is suggested that biomarkers of renal complications of Fabry disease are likely to be useful for diagnosis and to follow the natural disease progression or the effect of specific therapeutic interventions. Traditionally, globotriaosylceramide (Gb₃) in urine has been used to evaluate the effect of specific therapy, such as enzyme replacement therapy (ERT). Although urinary Gb₃ decreases significantly with ERT, it has not yet been shown to be a valid surrogate marker in treatment trials. We propose a detailed study of the nature and origin of Gb₃ combined with a prospective collaborative trial that combines Gb₃ changes with the effect of ERT on clinical nephrological outcome measures. Existing biomarkers such as general proteinuria/albuminuria or specific proteins such as N-acetyl-β-D-glucosaminidase should be evaluated along with novel proteomic or metabolomic studies for biomarker discovery using mass spectrometry or nuclear magnetic resonance. Standard scoring of all pathologic aspects of kidney biopsies may also be a promising way to assess the effect of therapy.


Fabry disease is rare and is typically associated with a marked increased risk of developing chronic renal insufficiency associated with progressive proteinuria (1). Efficacious therapy is expected to stop or at least slow the decline of kidney function, but its effect can only be ascertained over period of years in large patient cohorts. Therefore, biomarkers that respond relatively quickly to effective therapy and predict renal progression may be useful to follow individual subjects or groups of patients. Such biomarkers may be particularly useful for the care of female heterozygotes who are known to have a clinical course that is difficult to predict.

What Do We Know?

Globotriaosylceramide (Gb₃), the main substrate of the deficient α-galactosidase A in Fabry disease, is known to be increased in patients’ urine (2,3). It is consistently elevated in virtually all patients (male and female) when measured in random sample of whole urine or urine deposited on a filter paper (Whatman 903) (3,6,7). Urine levels are highest in patients with marked increased risk of developing chronic renal insufficiency associated with progressive proteinuria (1). Efficacious therapy is expected to stop or at least slow the decline of kidney function, but its effect can only be ascertained over period of years in large patient cohorts. Therefore, biomarkers that respond relatively quickly to effective therapy and predict renal progression may be useful to follow individual subjects or groups of patients. Such biomarkers may be particularly useful for the care of female heterozygotes who are known to have a clinical course that is difficult to predict.

One key question remains unanswered: Where does the Gb₃ biomarker come from? Urinary Gb₃ has its origin mostly in kidney tubular cells of the kidney and urinary collecting system. These assumptions are based on the presence of Gb₃ in lysosomes of renal tubular cells shed in the urine and an 80% reduction in urinary Gb₃ after transplantation and nephrectomy (11–13). Dense accumulation of Gb₃ has been observed in podocytes from Fabry disease patients, which could be partially reversed by long-term enzyme replacement therapy (ERT). This would indicate that the shedding of podocytes or even leakage through renal glomeruli of circulating Gb₃ may also be a significant source of this glycosphingolipid in urine (4,14).

Urinary Gb₃ has been used as a biomarker, but the clinical and research applications of this analyte are still controversial (9). Gb₃ is clearly useful as a diagnostic marker for individual patients and probably for screening for the disease in populations of patients at risk (2,6,15,16). It can be used as an indicator of the metabolic effect of infused α-galactosidase A for ERT (17,18). As soon as 2 wk after initiation of ERT, urinary Gb₃ levels decrease significantly (5) but may increase again when anti-α-galactosidase A antibodies develop (17,19). Thus, mea-
suring this glycosphingolipid may indicate the presence of such antibodies and help monitor patients during toleration efforts. However, there is thus far no published evidence that urinary Gb3 has prognostic value or that the degree of its decline during ERT can serve as a surrogate marker for the effect of specific therapy on the nephrologic clinical outcome in treatment trials. A recent study showed that 27 patients with the same missense mutation (A143P) had various clinical manifestations, such as cardiomyopathy (41% of the patients), left ventricular hypertrophy (11%), stroke, decreased renal function (7%), kidney transplant (4%) and the common clinical Fabry symptoms such as acroparesthesia, hypohidrosis, pain, angiokeratomas, and intolerance to heat, fever, and diarrhea (37%). The patients’ estimated GFR showed no correlation with urinary excretion of Gb3 (6).

There are other “general” biomarkers in urine that indicate glomerular or tubular abnormalities and are elevated in patients with Fabry disease. Total urinary protein and albumin excretion can be considered as important biomarkers in renal Fabry disease (1,20). The higher the proteinuria, the more rapid the decline in kidney function, whereas reduction in proteinuria guides the use of angiotensin converting enzyme inhibitors/angiotensin receptor blockers and is associated with significant stabilization of renal glomerular function (1,20,21). Therefore, proteinuria and albuminuria are examples of excellent and available biomarkers in Fabry nephropathy that predict the risk of progressive renal failure and indicate the effectiveness of therapy. N-acetyl-β-D-glucosaminidase and β-2-microglobulin are increased in urine of patients with Fabry disease (unpublished data). Uromodulin (Tamm–Horsfall glycoprotein) is abnormally processed in patients’ urine, with indication of normalization of processing with ERT, but no relationship to renal progression was reported (22). The abnormality of proteins is an indicator of tubular (particularly proximal) dysfunction and their role is likely to be similar to other proteinuria nephropathies such as diabetes (23). Other urinary proteins may serve as useful biomarkers. The utility of these biomarkers to predict the course of the disease should be studied.

Podocytes are terminally differentiated cells with a limited replication potential. Injuries that alter their structure and ultimately induce their detachment from glomerular basement membrane result in glomerular podocytopenia because the remaining podocytes may fail to restore the original cell number. The net consequence is the presence of podocytes in urine, a phenomenon called podocyturia. Low numbers of podocytes are usually excreted in the urine in the healthy state, whereas in some glomerular diseases podocyturia increases significantly (24), which renders this parameter a potentially valuable, noninvasive approach to monitor the progression of diseases such as focal segmental glomerulosclerosis, diabetic nephropathy, membranous and IgA nephropathy, and preeclampsia (25–27).

Podocytes detached from the glomeruli can be measured in the urine by cytological methods. Among the podocyte surface proteins podocalyxin is the most reproducible marker for staining of urine sediments, although this protein is also expressed on other cell types such as endothelial cells and parietal epi-thelial cells (14). Work is in progress to determine the appropriate urinary markers for monitoring podocyte excretion in different glomerular diseases. Podocyte count in urine sediments is a time-consuming procedure and it may be technically challenging to obtain reliable data because the reading is observer-dependent. However, recent studies have shown the feasibility of measuring mRNA of podocyte origin through quantitative reverse transcription-PCR of urine sediments (28,29). Currently, the evaluation of podocytes in the urine, although promising, cannot be considered a substitute for renal biopsy but could possibly constitute a tool for follow-up of an outpatient after biopsy-proven diagnosis.

What Can We Do with What We Do Know?

Urine Gb3

Until prospective studies demonstrate its usefulness as a surrogate marker for clinical efficacy of specific therapies for Fabry disease, urinary Gb3 levels can only be used as a diagnostic marker and a means to assess the metabolic effect of interventions such as ERT, pharmacologic chaperones, or substrate reduction therapy. Reduction in urinary Gb3 is likely to be correlated with reduction in tissue Gb3. However, tissue Gb3 measurement, either as lysosomal inclusions or biochemically as total Gb3, may not be a universal surrogate marker. In fact, patients who do not respond to ERT are presumed to have an equally large reduction in this biomarker as those whose decline in renal function is significantly slowed by ERT (18,30).

Proteinuria

This is the most important biomarker in Fabry kidney disease (1,30). Protein levels should be kept as low as possible. When elevated, protein levels should be brought down with angiotensin converting enzyme inhibitors/angiotensin receptor blockers to <0.5 g/24 h (20).

No other biomarker (other than serum creatinine) has been shown to be useful to follow renal Fabry disease.

Key Research Questions

Urinary Gb3

It is possible that podocyte shedding contributes to the total amount of urinary Gb3 and therefore the number of podocytes or podocyte-derived Gb3 may be measured in urine. Such markers may be more useful because they originate in the glomerulus—the part of the nephron most relevant to the GFR. The measurement of the ratio of nephrin or podocin mRNA factored for a kidney-specific reference gene mRNA in urine might enable distinguishing the fractions of Gb3 that are glomerular from those that are of tubular origin (28). Identification of isoforms of Gb3 in urine not present in glomerular filtrate may allow further focus on the “nephrogenous” glycosphingolipid fraction.

There is therefore a need for a collaborative prospective study of Gb3 in the urine at baseline and with ERT to correlate with clinical markers (e.g., GFR) and other markers of health of various parts of the nephron, including tubular involvement. It is important to state that the change in Gb3 has to be assessed in the context of confounders such as proteinuria and the use of
antiproteinuric medications. This may define the role of Gb₃ as a clinical outcome measure in treatment trials and in managing the individual patient.

**Urinary Proteomics**

Urine represents an attractive biofluid in clinical proteomics because it can be obtained in large amounts, with noninvasive procedures, and urinary peptides and proteins are relatively stable. Mass-spectrometry-based urinary proteins and peptide profiling are regarded as potential tools for clinical validation of urinary biomarkers (31). Despite the latest improvements in biomarker discovery, analysis of the urinary proteome is now faced with sequence identification of the biomarkers.

Albuminuria remains the best existing biomarker for most nephropathies, and Fabry nephropathy is no exception. Albuminuria may be supplemented by the identification of novel urinary proteins (single proteins or a combination) by mass spectrometry or other methods may be a means of biomarker discovery in Fabry disease (32). Preliminary unpublished data have already been generated. Isolation of exosome may allow identifying the coupling between some of the urinary proteins and Gb₃.

**Urinary and Plasma Metabolomics**

Similar approaches can be taken by looking at metabolites in biologic fluids by mass spectrometry (33) or nuclear magnetic resonance spectroscopy (32). In fact, the future of biomarkers may possibly be found in the developing area of metabolomics by doing quantitative metabolic profiling of many small molecules or metabolites (the metabolome) in biologic fluids. Metabolites fulfill the key criterion that they change rapidly in response to physiologic changes and may generate important information about biochemical pathways that are modified in Fabry disease and in treated patients. The profiling of metabolites in Fabry patients, including lipids, sugars, nucleotides, organic acids, and amino acids, may be useful because they serve as substrates and products in biochemical metabolic pathways. They may provide insight into the cardiovascular and/or renal aspects of Fabry disease. Moreover, metabolic fingerprinting can also be performed using an unbiased, global screening approach to classify samples on the basis of metabolite patterns or fingerprints that change in response to Fabry disease treatment or genetic perturbations with the ultimate goal to identify discriminating metabolites (34). A recent study described changes of fibrinolysis and angiogenesis factors in pediatric patients with Fabry disease after ERT by using differential isotope labeling with tandem mass spectrometry (35). How- ever, the limitation of the technique to provide relative but not absolute values of identified proteins does not allow understanding the contribution of such protein abnormalities to Fabry disease pathophysiology.

**Structural Analysis of the Kidney**

Systematic kidney biopsy scoring (International Study Group of Fabry Nephropathy) (36) and morphometry (37,38) are being developed. These methods, if validated, may be used to assess the effect of specific therapy.

**Other Biomarkers**

Metabolites related directly to the primary defect of Fabry disease such as lyso-Gb₃ can be evaluated in blood (39) or urine (unpublished data). More systemic biomarkers, including vascular ones, indicated that inflammation or growth factors should be evaluated (40,41).

Hopefully, the identification of easily measured biomarkers that reliably reflect disease progression and severity and thereby facilitate the evaluation of therapeutic responses by improved monitoring will improve the management of Fabry disease patients.

**Appendix**

Participants in the Symposium Session “Biomarkers of Fabry Nephropathy and progression” at the Official Satellite of the World Congress of Nephrology titled “Focus on Fabry Nephropathy: Biomarkers, Progression and Disease Severity”, held in Bergamo, Italy, on May 28th, 2009, included R. Schieffmann, Dallas, Texas; S. Waldek, Manchester, United Kingdom; A. Benigni, Bergamo, Italy; C. Auray-Blais, Sherbrooke, Quebec, Canada; M. Cuccurullo, Napoli, Italy; A. Kristler, Zurich, Switzerland; B. Najafian, Minneapolis, Minnesota; M. Rosenberg, Tartu, Estonia; L. Tartaglia, Foggia, Italy; and X. Zikou, Ioannina, Greece.

In addition to the above-named participants, Genzyme Corporation (1), Shire Human Genetic Therapies (1), and Amicus Therapeutics (2) employees were present at the meeting but had no role in the presentations and did not influence the outcome of the discussions.

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**References**