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Biomarkers in anderson-Fabry disease: what should we use in the clinical practice?

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Abstract

Major organ involvement in Anderson-Fabry disease (FD) is clinically silent for a long period and clinically heterogeneous; thus, it is difficult to identify the patients at increasing risk of a progressive disorder. Moreover, accumulating evidence suggests that early disease-specific treatment (DST) is safe and effective in preventing the progression of heart and kidney damage, with poorer results in patients with extensive myocardial fibrosis, advanced glomerulosclerosis, and/or heavy proteinuria. Therefore, biomarkers defining preclinical involvement, with a prognostic value and a correlation with response to treatment, are an urgent need in FD. Several types of biomarkers are recognized in FD, pertaining to total disease burden and specific organ involvement (central nervous system, heart, and kidney). Currently, plasma globotriaosylsphingosine (lyso-Gb3), cardiac and brain imaging, and albuminuria are recognized as the “gold standard” biomarkers of total disease burden or specific organ involvement in FD. However, severe globotriaosylceramide (Gb3) storage and organ damage may occur within the affected organs with minimal changes in these standard tests. Given the heterogeneity and rarity of the disease, the identification of new biomarkers is challenging. Several ways may be used to identify new biomarkers in FD, namely “omic” medicine, biomarkers identified in other pathological models similar to FD, and biomarkers linked to the pathophysiological pathways involved in FD. This article aims to review the clinical value of the available biomarkers in FD and give an overview of the research on new biomarkers.

Keywords: Anderson-Fabry disease, biomarkers, lyso-Gb3, cardiac imaging, albuminuria



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INTRODUCTION

Biological markers or biomarkers are defined, according to the National Institutes of Health Biomarkers Definitions Working Group, as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”^[1]. Biomarkers may have great value in several clinical applications, including their use as diagnostic tools, for staging or classifying the extent of the disease, disease progression and prognosis, predicting and monitoring clinical response to an intervention, and facilitating early evaluation of efficacy and safety in clinical trials^[1]. However, rigorous validation of the relationship between a proposed biomarker, disease activity, and outcome is of key importance^[2].

Candidate biomarkers for lysosomal storage disorders (LSDs) are mainly analytes and imaging techniques. Analytes may range from simple metabolites to complex proteins and, for LSDs, can be divided into two categories: molecules that accumulate in tissues and body fluids directly due to the enzymatic defect, and molecules produced by the cells in response to lysosomal storage^[3].

One of the most urgent needs in Anderson-Fabry disease (FD) is for reliable and validated biomarkers, ideally measured by non-invasive testing. This urgency is mainly related to the characteristics of and pitfalls in FD: diagnosis, determination of phenotype (classical vs. late-onset), evaluation of preclinical involvement, monitoring and assessment of treatment response.

The diagnosis of FD is based mainly on the enzymatic activity of α -galactosidase A and GLA gene sequencing, but both methods have limitations that need to be addressed. Due to random X chromosome inactivation, female patients may present significant residual enzymatic activity and about one-third may have α -galactosidase A activity within the normal range for the general population; thus, the diagnosis can only be reliably performed by GLA sequencing^[4]. Moreover, in male patients with residual enzymatic activity (> 5% of normal)^[5,6] definitive diagnosis of FD may only be confirmed by GLA gene mutation analysis^[7]. However, GLA gene sequencing may not provide a definitive diagnosis for several reasons: most GLA mutations found in FD patients are novel/“private”, there are various mutations of unknown significance, and routine sequencing can only identify a mutation in 97% of male patients with FD^[7,8]. Hence, reliable biomarkers may help diagnosis in these situations^[1].

Furthermore, FD is clinically heterogeneous (in presentation and rate of progression)^[9-12], with genetic factors and gender certainly contributing to this fact^[13,14]. However, the clinical picture may vary widely even in patients within the same family or with the same mutation, and thus other genetic, epigenetic, and environmental factors also contribute to clinical heterogeneity. Therefore, the identification of prognosis biomarkers and biomarkers capable of determining the likely phenotype (classical vs. late-onset) is paramount to the identification of patients at increased risk of a progressive disorder^[1].

FD is clinically silent for a long period; per example, severe storage material inclusions in podocytes and distal tubules, as well as segmental foot process effacement and nonspecific degenerative lesions, have been identified even at early stages of Fabry nephropathy, in pediatric and adult patients with minimal or no alterations in standard renal tests (namely glomerular filtration rate, albuminuria or proteinuria)^[15-20]. Although the optimal timing of disease-specific treatment (DST) beginning is not known, increasing evidence suggests that an early treatment strategy may be more effective in preventing cardiac and renal manifestations and major clinical events. However, currently, the European recommendations for DST initiation are based on functional or structural manifestations^[21]. Nonetheless, as mentioned above, there is a long clinically silent period before overt major organ manifestations, characterized by histological changes

(often irreversible lesions) or transcriptional profiles, whose detection depends on invasive procedures. Consequently, the identification of non-invasive biomarkers of preclinical involvement of the organs may have a profound impact on the treatment decisions, whether these biomarkers prove to have prognostic value^[1].

Finally, FD is a relatively slowly progressive disorder, with major events occurring mainly in adulthood. This fact constitutes a major difficulty in the design of clinical trials^[22-24], because long follow-up periods are required to demonstrate the benefits of any therapeutic intervention. Therefore, the identification of surrogate biomarkers of response to treatment, with rigorous correlations with clinical outcomes, is essential in the evaluation of the therapeutic strategies that are available or under development^[1].

FABRY DISEASE BIOMARKERS USED IN THE CLINICAL PRACTICE

Currently, there are no proper or well established/validated plasma or urinary biomarkers for FD. However, there are several biomarkers (imaging techniques and analytes [metabolites related to lipid abnormalities or proteins]) that are used in clinical practice with some limitations. These biomarkers may reflect the systemic disease burden or manifestations in a particular organ.

SYSTEMIC DISEASE BURDEN BIOMARKERS

Metabolites related to lipid abnormalities have been used as the main biomarkers of FD systemic burden, namely globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3).

Research of potential protein biomarkers for FD showed a lack of prominent plasma protein abnormalities. However, several biomarkers mainly related to inflammation and endothelial dysfunction have been studied, but not widely validated and, consequently, are not currently used in clinical practice^[1].

Globotriaosylceramide

FD is characterized by a disruption in glycosphingolipids metabolism, so lipid abnormalities have been studied as potential biomarkers. For a long time, the primary accumulating substrate Gb3 has been considered a surrogate marker for FD [Table 1], and its reduction in the endothelium has served as an indicator for the development and registration of enzyme replacement therapy (ERT) with agalsidase β ^[1,22,25].

Role in diagnosis and phenotype evaluation

Gb3 is not only found inside the cells, but it has been recognized for a long time that its concentration is increased in plasma and urine in FD male patients^[26]. Plasma and urine Gb3 values are more strikingly elevated in FD males and plasma Gb3 is elevated in only a small percentage of female patients (15%-30%), and urinary Gb3 may be within the normal range in 8%-13% and 12%-20% of male and female patients, respectively^[27,28]. There is a correlation between types of mutations and urinary Gb3 excretion, with patients with missense mutations, mainly those associated with late-onset phenotypes, presenting lower values, most of which within the normal range, even in male patients^[29-31]. Thus, the added value of Gb3 for diagnostic purposes is at least questionable^[1].

Role in clinical monitoring and assessment of treatment response

The correlation between plasma or urinary Gb3 and clinical manifestations and its added value to monitor progression of FD is also poor, without correlation with age, most of clinical parameters, and total disease severity (measured by Mainz Severity Score Index [MSSI])^[32]; however, plasma Gb3 is significantly higher in male patients with cerebral complications (compared with those without) and urinary Gb3 significantly correlates with estimated glomerular filtration rate (GFR), urine protein-to-creatinine ratio, and albumin-

Table 1. Systemic disease biomarkers used in clinical practice

Biomarker	Diagnosis	Phenotype	Clinical correlations	ERT monitoring	References
Gb3 (plasma/urine)	Not useful	Not useful	Poor: • Plasma Gb3 with cerebral complications (♂) • Urinary Gb3 with eGFR, PCR, and ACR	Decrease during ERT: • Correlation with clinical endpoints not established	[27,28,30,33,36]
Lyso-Gb3 (plasma)	Added value (cautious interpretations of results)	Added value (excellent diagnostic accuracy in discriminating between phenotypes and genders)	• ♀: LV mass and MSSl • ♂: WML	Decrease during ERT: • ♀: correlation with decrease in LV mass and HR to WML	[40,41,43,46,49,61]

ERT: Enzyme replacement therapy; Gb3: globotriaosylceramide; eGFR: estimated glomerular filtration rate; PCR: protein-to-creatinine ratio; ACR: albumin-to-creatinine ratio; LV: left ventricle; MSSl: mainz severity score index; WML: white matter lesions; HR: hazard ratio.

to-creatinine ratio (ACR)^[27,33]. Finally, there was significant decrease in plasma and/or urine Gb3 in patients treated under the clinical trials of both ERT preparations^[22,24,34,35]; however, the correlation between this decrease and the therapeutic outcome in terms of “intermediate” or “hard” endpoints is not established^[28,30,36]. Moreover, in one study, the occurrence of anti-ERT antibodies was accompanied by a blunted decrease in urinary Gb3, but the clinical significance of this finding remains unclear^[37]. The limitations of Gb3 in terms of predictive value for FD manifestations are not surprising, given that prominent Gb3 has been noted in placental tissues of FD male patients^[38,39], with the onset of clinical complications occurring only several years later; thus, as mentioned above, other factors in addition to Gb3 may participate in pathogenesis^[1].

Globotriaosylceramide

Given the limitations of Gb3 as a biomarker, research on Gb3 metabolites identified a product of Gb3 deacetylation, globotriaosylsphingosine (lyso-Gb3), which is hydrophilic and highly diffusible and whose plasma levels in FD patients are markedly increased (exceeding those of controls by more than one order of magnitude and most prominent in male patients) [Table 1]^[1,40].

Role in diagnosis and phenotype evaluation

In male patients with classical phenotype, several studies showed very high values of plasma lyso-Gb3^[30,40-43]; however, even hemizygotes with late-onset phenotypes presented increased lyso-Gb3 levels (though in lower magnitude than patients with classical phenotype)^[1,31,44-52].

Regarding female patients, the classically affected heterozygotes usually present with increased plasma lyso-Gb3 levels (in a magnitude similar to late-onset male patients)^[43,47], contrary to female patients carrying mutations associated with late-onset phenotypes (e.g., p.F113L, p.N215S or IVS4 + 919G>A), where plasma lyso-Gb3 is often normal^[31,44-46,48,50,51]. Despite this limitation of lyso-Gb3 in the diagnosis of female patients, the sensitivity of plasma lyso-Gb3 is much higher, in comparison with α -galactosidase A activity, to identify female patients with FD^[43,53]. Additionally, this sensitivity may be further increased using the α -galactosidase A activity to plasma lyso-Gb3 ratio^[54].

Moreover, plasma lyso-Gb3 presented excellent diagnostic accuracy in discriminating between classical and late-onset phenotypes in male and female patients [Figure 1], with an area under the curve of 0.990 and 0.954, respectively^[46,49].

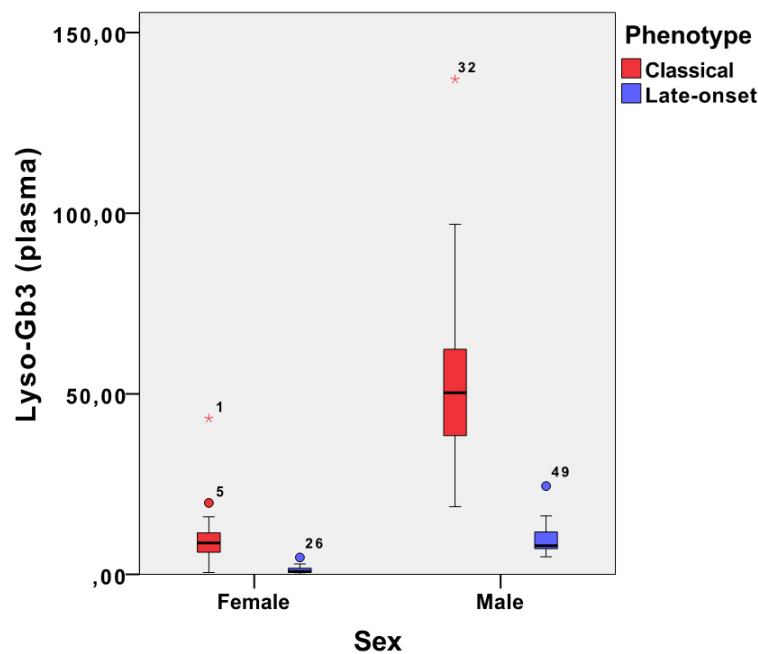


Figure 1. Plasma lyso-Gb3: sub-analysis by gender in male and female patients with Fabry disease^[46]. Lyso-Gb3: Globotriaosylsphingosine.

However, the discriminative power of plasma lyso-Gb3 in male and female patients with variants of unknown significance, like p. R118C, p.R112H, and p.P60L, seems limited, as lyso-Gb3 is not increased in this group of patients (even in patients with histological demonstration of Gb3 storage in podocytes)^[31,41,45,55,56]. Nonetheless, recently, nano-liquid chromatography-tandem mass spectrometry (a technique enabling the detection of extremely low concentrations of lyso-Gb3, with greater sensitivity than conventional techniques), in patients with variants of unknown significance (p.R112H and p.M296I), demonstrated that lyso-Gb3 was lower than in classical and late-onset phenotypes FD patients having other variants, but higher than in those with functional variants (p.E66Q) and healthy subjects^[1,57].

The performance of urinary lyso-Gb3 as a diagnostic tool seems similar to that of plasma lyso-Gb3, with only a minority of patients excreting undetectable amounts and with patients presenting missense mutations or mutations associated with late-onset phenotypes having significantly lower excretion of lyso-Gb3^[33,47]. Thus, lyso-Gb3 seems to be a promising diagnostic biomarker, with added value for diagnosis in specific situations, but evaluation of pathogenicity of mutations based solely on this parameter should be cautious^[1].

Role in clinical monitoring

Various correlations have been found between plasma lyso-Gb3 and clinical manifestations. However, as stated previously, given the large differences in plasma lyso-Gb3 levels between male and female genders or classical and late-onset phenotypes, a sub-analysis by these subgroups is paramount in order to avoid the confounding factor of disease severity (higher in males and classical phenotype) and to understand the true clinical correlations and prognostic value of plasma lyso-Gb3 in single patients, for whom the gender and phenotype are already known; thus, in a study with analysis by these sub-subgroups, only significant clinical correlations were found between plasma lyso-Gb3 in classical males and indexed LV mass, as well as between plasma lyso-Gb3 in classical females and total MSSI [Table 2]^[46].

Table 2. Correlation between lyso-Gb3 and clinical variables in male and female patients (sub-analysis by phenotype)^[46]

	All patients (n = 73)	Males Classical (n = 18)	Late-onset (n = 11)	Females Classical (n = 29)	Late-onset (n = 15)
Age	0.001	0.325	-0.247	0.309	0.082
Age at ERT initiation (n = 50)	-0.466*	0.088	-0.071	0.230	-0.100
MSSI	0.538*	0.458	-0.251	0.441*	0.404
FIPI	0.383*	0.424	-0.196	0.259	0.363
Indexed LV mass	0.353*	0.776*	-0.327	0.303	0.045
NT-pro BNP (n = 24)	0.247	-	-	-	-
Albuminuria	0.317*	0.079	-0.600	0.133	0.380
eGFR	-0.168	-0.066	0.345	-0.125	0.015

* $P < 0.05$; ρ for correlation between variables and plasma lyso-Gb3. ERT: Enzyme replacement therapy; MSSI: Mainz severity score index; FIPI: Fabry international prognostic index; LV: left ventricular; NT-pro BNP: amino-terminal fragment of the prohormone of brain natriuretic peptide; eGFR: estimated glomerular filtration rate.

In male patients with classical phenotype, lyso-Gb3 does not increase with age^[49] (with markedly increased values even in neonates, which increases rapidly during the first months of life)^[58] and consequently does not correlate strictly with disease severity indexes or clinical manifestations, except for a significant correlation with the presence of white matter lesions^[1,40,41]. One study described a significant correlation between plasma lyso-Gb3 and MSSI in male patients, but there was no discrimination between patients with classical and late-onset phenotypes^[43].

Contrariwise, in late-onset phenotype male patients (presenting p.N215S variant), there was a significant increase in plasma lyso-Gb3 with age and it presented significant correlations with left ventricular (LV) mass and GFR^[48]; these results were in contrast with the ones of other studies, showing no correlations between plasma lyso-Gb3 and age, clinical manifestations or disease severity indexes in male patients with late-onset phenotypes [Table 2]^[46].

In females, lyso-Gb3 tends to increase progressively with age^[49] and significant correlations have been found between lyso-Gb3 and MSSI, LV mass, and carotid intima-media thickness^[40,41,59]. Contrariwise, another study found no correlation between plasma lyso-Gb3 and MSSI in females^[43].

There are no well-established correlations between plasma lyso-Gb3 and kidney function parameters, but a significant correlation between urinary lyso-Gb3 and urine protein-to-creatinine ratio or ACR has been observed; however, this correlation was observed in a cohort of male and female patients and no adjustment for gender was reported^[1,33]. Another study also reported a significant correlation between plasma lyso-Gb3 and serum creatinine, protein-to-creatinine ratio or indexed LV mass in multivariate analysis adjusted for gender and phenotype^[49].

As lyso-Gb3 seems to be a risk factor directly implied in FD pathogenesis, lifetime exposure to plasma lyso-Gb3 (estimated product of lyso-Gb3 concentration by age) should correlate better with disease (mainly in male patients, showing very high values from birth) than the current lyso-Gb3 level. Therefore, both in male and female patients, plasma lyso-Gb3 exposure is significantly correlated with disease severity, clinical manifestations, and the cold detection threshold and thermal sensory limen of the upper limb (both signs of characteristic FD small fiber neuropathy)^[1,41,48,60]. However, as FD is a storage disorder with a progressive accumulation of substrates along patients' life, older patients tend to have more severe manifestations; thus,

the analysis of plasma lyso-Gb3 over a lifetime is biased by the age effect on disease severity.

Therefore, the correlations between plasma lyso-Gb3 and clinical variables are limited, with contradictory results among studies and most of the studies not reporting a sub-analysis by gender and phenotype, biasing the results and precluding comparisons between them. Moreover, no proper longitudinal studies with repeated measurements of plasma lyso-Gb3 are available within the literature in order to evaluate its prognostic value, and even patients with plasma lyso-Gb3 within the normal range may present clinical events^[51].

Role in the assessment of treatment response

Lyso-Gb3 decreases significantly with any of the three available ERTs (with a more pronounced decrease in classically affected males), but does not reach normal values, even in female patients, independently of ERT preparation^[30,40,42,52,61-63]. Moreover, in classically affected males developing anti-ERT antibodies, reduction of plasma lyso-Gb3 is relatively poor^[40,42,52,61,64], mainly in patients treated with agalsidase α (with a significant difference between patients with and without anti-drug antibodies)^[60]. One study reported that classically affected males starting treatment before 25 years of age have a greater decrease in plasma lyso-Gb3 compared with patients starting ERT later in life, with this difference remaining significant even after adjustment for plasma lyso-Gb3 at baseline, ERT preparation, and ERT dose. Furthermore, the patients starting ERT at an earlier age presented a trend for less formation of anti-ERT antibodies, and there were fewer clinical events in the group of patients treated earlier, but the clinical meaning of the former finding is unclear due to an age bias^[65].

In patients treated with the *chaperone* migalastat, there is also a significant decrease in plasma lyso-Gb3 in the first six months of therapy in treatment-naïve patients^[66,67]. Moreover, in patients previously treated with ERT, plasma lyso-Gb3 remained stable after switching from ERT to migalastat^[67-70]. In patients treated with migalastat but non-amenable *in vivo*, there is no decrease or increase in plasma lyso-Gb3 in treatment-naïve patients or patients switching from ERT, respectively^[66,68,71,72]. However, in a single study, no significant correlation was found between plasma lyso-Gb3 change during treatment and the increase in leucocyte activity after migalastat initiation^[71].

The reduction in plasma lyso-Gb3 during ERT was found to correlate with the correction of LV mass in female patients and with a lower hazard ratio for developing new cerebral white matter lesions in male and female patients^[61]. However, in another longitudinal study, with a subanalysis by gender and phenotype and comprising mainly late-onset phenotype patients, there was no correlation between the change of plasma lyso-Gb3 and the change of LV mass in both classical and late-onset FD patients^[73]. This absence of correlation between plasma lyso-Gb3 and treatment outcomes was further confirmed in a large cohort of FD patients, showing that plasma lyso-Gb3 before treatment initiation, plasma lyso-Gb3 absolute decrease, or plasma lyso-Gb3 relative decrease did not predict the risk of event during ERT^[74]. The same results, with no correlation between changes in lyso-Gb3 and LV mass, GFR, or clinical events, were found for patients treated with migalastat^[75]. These results are in conflict with a single study showing a correlation between baseline lyso-Gb3 and the risk of an event during a median follow-up of 68 months, but all but one event occurred in classical phenotype patients, and when baseline lyso-Gb3 was added to a multivariable logistic regression model containing age, sex, phenotype and ERT as other covariates to identify the risk of an event, no significant improvement was found^[76]. Thus, the value of plasma lyso-Gb3 to monitor response to DST seems at least questionable, and its correlation with clinical outcomes clearly needs further investigation in larger cohorts of FD patients.

FABRY DISEASE CARDIOMYOPATHY BIOMARKERS

Currently, imaging techniques are the most commonly used biomarkers of cardiac injury in FD, but some biochemical biomarkers are also used in clinical practice [Table 3].

Troponin

Role in clinical monitoring

Cardiac troponins are well-validated biomarkers of cardiomyocyte injury. New generation/high-sensitivity assays for cardiac troponin T enable the identification of minimal cardiac injury and have been associated with poor outcomes in cardiomyopathies other than ischemic heart disease^[77,78]. Two studies evaluating the performance of troponin I as a biomarker of FD cardiomyopathy have shown that this analyte was elevated in 21%-37% of the patients, with a very high diagnostic accuracy for LV hypertrophy or late gadolinium enhancement (LGE) in cardiac magnetic resonance imaging (MRI)^[79,80]. Comparable results were obtained with high-sensitivity troponin T^[81-85] and one study showed a strong correlation with T2 in basal inferolateral wall^[86]; in a retrospective analysis, over a follow-up period of 3.9 years, patients with elevated troponin at baseline had significantly increasing replacement fibrosis^[81].

Role in the assessment of treatment response

High-sensitivity troponin T remained stable in the first 12 months after ERT initiation, while increased in untreated patients or in treated patients with advanced cardiomyopathy^[87]. This biomarker also remained stable within the first 18 months after migalastat initiation^[88].

Brain natriuretic peptide

Role in clinical monitoring and assessment of treatment response

Brain natriuretic peptide and the N-terminal fragment of its prohormone (NT-proBNP) have an established role in determining the diagnosis and prognosis of heart failure^[89,90]. NT-proBNP was evaluated as a biomarker of early cardiac involvement in FD, showing significant correlations with parameters of diastolic dysfunction and LV wall thickness^[91,92]. Other studies showed that NT-proBNP is significantly higher in patients with LGE in cardiac MRI and presented a significant correlation with the amount of LGE and the T2 in the basal inferolateral wall and an inverse correlation with native T1^[83,86,93]. NT-proBNP remained stable after ERT or migalastat initiation^[87,88].

Echocardiography

Nonetheless, cardiac imaging techniques are the mainstay in the assessment of FD cardiomyopathy. Conventional two-dimensional and Doppler echocardiography is the standard imaging tool in the identification and staging of cardiac involvement in FD disease, but it is not suitable for detecting subtle myocardial dysfunction in the early course of FD cardiomyopathy^[94,95]. Therefore, advanced echocardiography techniques, such as tissue Doppler imaging (TDI) and strain and strain rate (SR) speckle-tracking based echocardiography have been used in early cardiac involvement that precedes overt LV hypertrophy and appearance of replacement myocardial fibrosis. Recently, an echocardiographic-based staging of FD cardiomyopathy was proposed and was demonstrated to have clear prognostic value. FD cardiomyopathy was categorized into four stages: stage 0 (no cardiac involvement); stage 1 (LV hypertrophy); stage 2 (left atrium enlargement); stage 3 (ventricular impairment, defined as LV ejection fraction < 50% or E/e' ≥ 15 or TAPSE < 17 mm)^[96].

Tissue Doppler imaging

There are several studies establishing the added value of TDI in the early detection of FD cardiomyopathy^[97-103]. In the first study on the evaluation of TDI in FD cardiomyopathy, it was reported that echocardiography did not show any difference between FD patients without LV hypertrophy and controls

Table 3. Fabry disease cardiomyopathy biomarkers used in clinical practice

Biomarker	Preclinical evaluation	Clinical correlations	ERT monitoring	References
Cardiac troponin	Not useful	<ul style="list-style-type: none"> • LV wall thickness • LGE volume in cardiac MRI • T2 in basal inferolateral wall 	Remained stable during DST	[79,80,86-88]
NT-proBNP	Not evaluated	<ul style="list-style-type: none"> • Diastolic dysfunction parameters and LV mass • T2 in basal inferolateral wall • Inverse correlation with T1 	Remained stable during DST	[83,86-88,91-93]
Echocardiogram (TDI)	Superior to conventional echocardiography in the detection of early cardiac involvement	LV wall thickness	ERT successfully prevented the appearance of abnormal TDI velocities	[99-101]
Echocardiogram (speckle-tracking)	Better sensitivity than TDI in detecting early diastolic dysfunction	<ul style="list-style-type: none"> • LGE in cardiac MRI • Functional status • Risk of cardiovascular events • Risk of WML 	ERT improves: <ul style="list-style-type: none"> • Systolic strain and strain rate (?) • LA peak positive strain 	[93,105,106,108,109, 118-120,122,123]
Cardiac MRI (LGE)		LGE amount correlates with: <ul style="list-style-type: none"> • LV mass • Regional myocardial function • Risk of ventricular arrhythmias 	Not useful: <ul style="list-style-type: none"> • LGE amount increase during ERT • Major predictor of response to ERT 	[82,93,121,124,143]
Cardiac MRI (T1 mapping)	Characteristic decrease in native T1	Staging of FD cardiomyopathy in 4 phases	Improvement during DST, mainly in earlier stages	[83,85,87,88,146, 150]

ERT: Enzyme replacement therapy; HCM: hypertrophic cardiomyopathy due to sarcomere protein gene mutations; LV: left ventricle; LGE: late gadolinium enhancement; MRI: magnetic resonance imaging; DST: disease-specific treatment; NT-proBNP: N-terminal fragment of the prohormone of brain natriuretic peptide; TDI: tissue Doppler imaging; WML: white matter lesions; LA: left atrial; FD: Fabry disease.

in conventional parameters of diastolic dysfunction, but there was a significant decrease in myocardial systolic and diastolic velocities, even in FD patients without LV hypertrophy (although of lesser magnitude than in patients with overt LV hypertrophy)^[100]. These results were further confirmed in several cohorts of patients^[87,88,97-99,101,103]. Abnormalities of systolic velocities in the right ventricle (RV), measured by TDI, have been recently described^[104].

Furthermore, it was identified a significant inverse correlation between systolic mitral annular velocity (S') or early diastolic mitral annular velocity (E') and interventricular septum (IVS) or left ventricle posterior wall (LVPW) thickness^[101]. The diagnostic accuracy of TDI variables in detecting myocardial fibrosis was also evaluated, with both septal and lateral E/E' ratios, an estimate of left ventricular end-diastolic pressure, presenting high diagnostic accuracy in predicting the presence of LGE in cardiac MRI^[93].

Regarding treatment effect, agalsidase α successfully prevented the appearance of abnormal TDI velocities in the group of patients with normal echocardiograms at baseline^[102]. It was concluded that reduction in myocardial contraction and relaxation velocities in TDI are detectable before the development of LV hypertrophy or even abnormalities in the traditional parameters of diastolic function, thus enabling the recognition of preclinical cardiac damage.

Strain and strain rate speckle-tracking

New tools like strain and SR speckle-tracking based echocardiography (a TDI-derived technique) enable measurement of myocardial systolic and diastolic strains, which seem superior and more sensitive than myocardial velocities (measured by TDI) in quantifying changes of myocardial systolic function, because they are less influenced by overall cardiac motion^[105].

Decreased systolic strain and SR of the LV have been reported in FD patients, compared with healthy controls^[106-109]. These alterations in myocardial systolic strains are detectable even in the early stages of FD cardiomyopathy: there is a significant reduction in both global systolic longitudinal and circumferential strains, as well as an absence of the normal regional base-to-apex circumferential strain gradient, even in the subgroup of patients without LV hypertrophy^[107,108,110-112]. Global longitudinal strain, mainly basal longitudinal strain, also correlated with LV mass, T1 mapping, or even the risk of “de novo” atrial fibrillation, major cardiovascular events, or stroke^[110,111,113]. RV strain is also impaired in FD patients, but only in the hypertrophic stage^[114] and may be lower than in patients with HCM^[115].

Decreased LV diastolic strain and SR were also reported in FD^[106,109], even in the early phases of FD cardiomyopathy (patients without LV hypertrophy) and with a higher sensitivity for detecting diastolic dysfunction compared to using TDI for early diastolic velocities^[109]. Left atrial (LA) systolic and diastolic strain and SR are also decreased in FD patients, with a significant decrease in systolic SR even in patients without LV hypertrophy^[116-118]; LA strain parameters are associated with atrial fibrillation and stroke^[119] and peak atrial longitudinal strain also showed an inverse correlation with white matter lesions (WML)^[120,121].

Speckle-tracking based echocardiography may also be a non-invasive tool for the detection of myocardial fibrosis in FD^[122,123]. Global longitudinal systolic strain is lower in patients with LGE in cardiac MRI, with a significant correlation between global longitudinal systolic strain and the amount of LGE; furthermore, segmental strain values are particularly decreased in the LGE positive basal posterior and lateral segments^[93,110,123]. Moreover, LV, RV, and LA strains are inversely linked to the heart failure functional class^[106].

The effect of ERT in myocardial systolic and diastolic strains is still controversial: in a study with agalsidase β , there was a significant increase in both longitudinal and radial peak systolic SR and systolic strain after 12 months of treatment^[124]; while in another study, in patients treated with ERT for a mean period of 3.1 years, there was no change in global systolic longitudinal or circumferential strain^[107]. The improvement in systolic SR may be influenced by the presence and amount of myocardial fibrosis at ERT initiation, with a study showing a significant increase in radial systolic SR occurring only in the subgroup of patients without fibrosis; systolic SR remained essentially unchanged in patients with mild fibrosis, but rather decreased in the severe fibrosis subgroup^[125]. ERT also seems to improve LA function by an increase in LA peak positive strain^[119].

Magnetic resonance imaging

Cardiac MRI plays a critical role in the differential diagnosis of cardiomyopathies and is the diagnostic standard for assessing global and segmental cardiac morphology and function, with high spatial resolution and low observer variability, in patients with FD^[126]. In patients under ERT, cardiac MRI can reliably evaluate its effects on LV mass in FD patients^[23,122,124,127-130].

Late gadolinium enhancement

LGE imaging techniques using cardiac MRI are the gold standard for non-invasive detection of focal replacement fibrosis in the myocardium^[131].

Between 31% and 77% of patients with FD disease may present LGE, with a characteristic mid-myocardial distribution (sparing the subendocardium) in the inferolateral basal and mid basal segments of the LV wall, that seems to be specific to FD cardiomyopathy^[82,83,93,122,125,131-136]. Additionally, in FD, LGE was correlated with histologic findings (myocardial collagen deposition) from autopsy^[137]. A greater proportion of male patients present larger amounts of LGE in cardiac MRI^[83,131,133]; it was reported previously that only female patients might present LGE with normal LV mass^[138], but recent reports showed that this is also true for some male patients^[82,83,133,139].

Several studies have reported a significant correlation between the amount of LGE and LV mass^[93,122,131,133,140,141], and LGE correlates with abnormalities in regional myocardial function assessed by speckle-tracking imaging studies^[93,122,123,135,138,142]. However, the sensitivity of LGE to detect early cardiac involvement, as expected due to the identification of irreversible replacement fibrosis, is limited: LGE is uncommon in patients showing only TDI abnormalities (without LV hypertrophy)^[93,143].

Cardiac fibrosis, evaluated by LGE in cardiac MRI, tends to increase progressively, mainly in patients with larger LGE volumes at baseline, even in patients with stable LV mass^[82]. Moreover, the annual increase in LGE during the follow-up is an independent predictor of malignant ventricular arrhythmias, with sudden cardiac deaths only occurring in the group of patients with LGE^[144]. Other studies confirmed that the presence and the extent of LGE, as well as maximal wall thickness, are associated with a greater risk of adverse cardiac events in FD patients^[145,146].

ERT does not seem to have any effect on LGE: the amount of LGE significantly increased in patients treated for 12 months with agalsidase β (no patients without LGE at baseline developed LGE during ERT), and LV mass only significantly decreased in patients without LGE at baseline^[122]. These results were further confirmed with longer treatment periods, also showing some patients developing LGE even during treatment with ERT^[144]. Moreover, as previously mentioned, the presence of LGE is one of the most important predictors of response to ERT in terms of myocardial function and exercise capacity^[125]. Similar results were found with migalastat, with no patients without LGE at baseline developing LGE during treatment, but with an increase in LGE in patients who initially present LGE^[147].

T1 mapping

A novel technique, T1 mapping, enables the measurement of native myocardial T1 (non-contrast myocardial T1) and T1 after administration of gadolinium contrast. Native T1 allows for a better characterization of the myocardium content, with increased values in the setting of fibrosis, edema, or amyloid deposits and decreased values in iron overload or lipid storage. Measurement of T1 with extracellular gadolinium-based contrast agents gives additional information about the extracellular volume fraction and has been studied for assessment of diffuse fibrosis, with good histological correlations^[148]. However, in FD, it has been more extensively studied as an imaging biomarker for early detection of cardiac involvement and for distinguishing FD from other etiologies of concentric remodeling and hypertrophy (due to lipid storage, yielding low native T1)^[149], rather than for evaluation of diffuse fibrosis^[150-154].

Very high (> 95%) diagnostic accuracy of native T1 in distinguishing between FD cardiomyopathy and other forms of cardiomyopathy has been reported^[85,152,153,155,156]. Septal native T1 is low in around 90% of the

patients with LV hypertrophy and in about 50% of the patients without LV hypertrophy (this subgroup had lower global longitudinal strain by speckle tracking and higher LV filling pressure), with lower values in male patients^[83,84,136,151]. In pediatric age, the native T1 is within the normal range, but in the pre-hypertrophic phase, it falls linearly with increasing age, and during adulthood, this decrease is less pronounced, although still significant; in the phase of overt hypertrophy, this correlation reverses (native T1 increases with the indexed LV mass) in male patients (remaining below the lower limit of normal) and the two variables become non-related in female patients^[83]. These data suggest that low T1 is a possible early marker of cardiac involvement and 3/4 phases of myocardial involvement are identified: phase 1: no involvement (early accumulating phase); phase 2: low T1 and early myocardial dysfunction (late accumulating phase); phase 3: LV hypertrophy with low T1 (characterized by inflammation, myocyte hypertrophy, and limited LGE); phase 4: “pseudonormalization” of T1, fibrosis, and heart failure (as extensive fibrosis and scarring present high T1 values)^[83,151].

A significant inverse correlation between native T1 and high-sensitivity troponin or plasma lyso-Gb3 was identified^[84,157,158]. Only very limited data are published about the prognostic value of native T1; one study reported that native T1 at baseline was significantly associated with disease progression (measured by FASTEX scale) at 12 months, with 100% of the progressing patients presenting low native T1, in comparison with only 53% of patients with no clinical progression^[159].

The effect of DST on native T1 was assessed in few studies, showing a trend for an increase during treatment with either ERT or migalastat, mainly in younger and less severely affected FD patients^[87,88,147].

T2 mapping

Only few studies evaluated T2 mapping, a biomarker of myocardial edema (related to inflammation), in FD, but no difference between FD patients and healthy controls, as well as no correlation with LV mass or left atrium volume, was found^[159]. However, T2 in LGE areas is significantly higher in comparison with patients with HCM or ischemic heart disease, and T2 in basal inferolateral wall is associated with GLS impairment, is the strongest predictor of troponin, and predicts clinical worsening after 1 year^[85,86].

Other imaging techniques

Other imaging techniques have been used to identify inflammation in FD cardiomyopathy. Hybrid techniques, such as positron emission tomography (PET)-MRI, have been studied for this purpose. These studies have revealed higher T1 native in the segments exhibiting higher ¹⁸F-fluorodeoxyglucose uptake, and a higher coefficient of variation in the isotope uptake has been associated with worse global longitudinal systolic strain measured by echocardiography^[160,161].

Cardiac scintigraphy is a useful tool to evaluate myocardial perfusion and energy metabolism. In FD, studies showed that energy depletion was associated with cardiac events^[162] and that sympathetic neuronal damage may precede myocardial damage, e.g., fibrosis^[163,164].

FABRY DISEASE NEPHROPATHY BIOMARKERS

Estimating glomerular filtration rate

Reproducible and accurate estimates of renal function are essential in the management of FD. There are limitations in all current equations to estimate GFR, but Modification of Diet in Renal Disease (MDRD) equation^[165] is not validated in patients with higher GFR (knowing that a hyperfiltration stage has been described in FD nephropathy) and seems less accurate than Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI_{creatinine2009}) equation^[166]. Thus, CKD-EPI_{creatinine2009} equation is the recommended

equation to estimate GFR in adults with FD^[167,168]. However, given that measured GFR by iothexol plasma clearance and isotopic methods is more accurate, depending on local availability, the precise measurement of the GFR is recommended for FD patients if the estimated GFR is $> 60 \text{ mL/min/1.73 m}^2$ ^[168].

Cystatin C was proposed as a reliable marker of renal function [Table 4]^[169]. Cystatin C has been compared with creatinine in the evaluation of renal function in a cohort of patients treated with agalsidase α for 4 years, with no significant change in creatinine or in creatinine-based estimated GFR, but with a significant increase (just after only one year of ERT) in cystatin C and a concurrent decrease in estimated GFR by Hoek equation^[170] (using cystatin C values). The authors concluded that cystatin C was an early marker for the decline of GFR, but no gold standard precise measurement of GFR was used to support this conclusion^[171]. This finding was further corroborated by another study^[92].

Cystatin C-based estimation of GFR was compared with measured GFR in a relatively small validation study (including several cystatin and creatinine-based equations). In contrast to that previously mentioned, Hoek equation was less accurate than CKD-EPI_{creatinine2009} equation in detecting GFR decline during ERT, but Stevens' equation^[172] (a creatinine and cystatin C-based formula) was the one that most closely approximated the measured GFR^[173]. However, Stevens' equation development was based on serum cystatin C assays in adults that were not traceable to standard reference material. Consequently, it is no longer recommended by international chronic kidney disease (CKD) guidelines^[167].

Albuminuria/proteinuria

Role in the identification of preclinical involvement

Total urinary protein and albumin excretion can be considered important biomarkers in FD nephropathy [Table 4]. Proteinuria ($> 150 \text{ mg/day}$) and albuminuria A2 are usually the first signs of renal involvement^[174-177]. The sensitivity of albuminuria seems superior to that of total urinary protein excretion in detecting early renal involvement in FD^[178]. Thus, albuminuria remains the best existing marker to detect early renal involvement, but the relevance of albuminuria A2 as a biomarker is largely based on validation studies in the earlier stages of other nephropathy models. Moreover, in FD, nephropathy albuminuria may result not only from glomerular damage of the filtration barrier, but also from tubular involvement with decreased reabsorption of filtered albumin.

However, the usefulness of albuminuria and/or proteinuria in identifying incipient FD nephropathy is questionable. Although there is a significant correlation between urinary protein excretion rates and foot process width and fractional volume of Gb3 inclusions in the podocytes^[16], FD nephropathy is clinically silent for a long period and significant histological changes (including nonspecific degenerative lesions) may occur without pathological albuminuria and/or proteinuria^[15-20]. In incipient stages of FD nephropathy, tubular reabsorption of albumin may overcome its increased excretion, limiting the sensitivity of this biomarker.

Prognostic value and role in the evaluation of treatment response

Furthermore, proteinuria also seems to play an important role in FD nephropathy progression, even in patients treated with ERT, because it is an independent risk factor affecting the extent of renal decline and is one of the determinants of the success of ERT^[12,178-185]. For both genders, the proportion of patients with overt proteinuria ($> 300 \text{ mg/day}$) and its magnitude and the prevalence of nephrotic range proteinuria are higher with more advanced CKD stages^[12,178]. Moreover, comparing the GFR decline stratified by baseline proteinuria, higher baseline proteinuria levels were associated with more rapid GFR decline, but the patients with higher baseline proteinuria were also older and with lower baseline GFR^[12,179]. Notwithstanding this, in

Table 4. Fabry disease renal biomarkers used in clinical practice

Biomarker	Diagnosis	Clinical correlations	ERT monitoring	References
Cystatin C	Better diagnostic accuracy than creatinine in detecting early renal involvement	Better correlation of cystatin C and creatinine-based equations with measured GFR (?)	More sensitive than creatinine in detecting a minor decline in GFR during ERT (?)	[92,169,171, 172]
Proteinuria albuminuria	Earlier biomarker of FD nephropathy (limitations)	Good correlation with GFR decline (there are several patients with CKD stage ≥ 3 without overt proteinuria)	Does not respond to ERT	[15-20,177-184]

ERT: Enzyme replacement therapy; GFR: glomerular filtration rate; FD: Fabry disease; CKD: chronic kidney disease.

one study, a regression model for GFR slope retained proteinuria as the most important indicator of renal disease progression in adult FD patients^[179]. Therefore, there seems to be an influence of proteinuria in nephropathy progression, although the magnitude of this influence is not well established, as several studies, with both male and female patients, found no relationship between the degree of proteinuria and the rate of GFR decline, and in one study, 11% of the male and 28% of the female patients with estimated GFR < 60 mL/min/1.73 m², presented no overt proteinuria^[9,10,178]; this seems also true for albuminuria, with one study showing a significant correlation between albuminuria and GFR in male patients, but not in females^[186].

Finally, ERT does not seem to reduce proteinuria (mainly in male patients), so this biomarker does not serve as a good indicator of response to ERT.

Histological lesions

It is well known that substrate storage occurs in all renal cells, leading to progressive nephropathy characterized by nonspecific degenerative lesions, namely mesangial widening, segmental and/or global glomerular sclerosis, tubular atrophy, and interstitial fibrosis^[15,187-190]. These well-known histological findings in advanced FD nephropathy contrast with the limited knowledge about the histology of patients with incipient nephropathy. Thus, histological biomarkers have been used in clinical practice to assess the prognosis of each individual patient.

Role in the identification of preclinical involvement

Lipid deposition-related lesions are characteristic of FD nephropathy, appearing as vacuoles or inclusions, according to the applied technique. The characteristic severe inclusions in podocytes and distal tubules, as well as segmental foot process effacement, have been shown even at early stages of Fabry nephropathy in pediatric and adult patients with minimal or no alterations in standard renal tests^[15-20]. Moreover, mesangial and endothelial cell inclusions, as well as nonspecific degenerative lesions, such as mesangial widening, glomerulosclerosis, tubulointerstitial fibrosis, and arteriopathy, have also been described in this group of patients^[15,17,19]. These data confirm that clinically silent deposition of Gb3 begins in early childhood, long before overt FD nephropathy, with a wide variation in the individual progression of glomerulosclerosis and interstitial fibrosis and development of end-stage renal disease^[12].

Prognostic value

The importance of these specific pathological findings as potential surrogate markers for the progression of renal dysfunction is uncertain and needs to be further studied longitudinally. In contrast to semi-quantitative scoring systems for intracellular Gb3 inclusions, using light microscopy, that has failed to show any correlation with age, proteinuria, and GFR^[9,15,17,191], a small study with 14 FD patients, using quantitative stereological electron microscopy methods, has shown significant correlations between podocyte Gb3 fractional volume of inclusions by cytoplasm or foot process width and age or proteinuria^[16]. Conversely,

chronic nonspecific glomerular and tubulointerstitial lesions seen in kidney biopsies seem to correlate better with the natural history and manifestations of FD nephropathy (GFR and proteinuria)^[9,15,191,192].

Role in the evaluation of treatment response

Histological biomarkers have also been used as surrogate endpoints to assess the response of FD to DST, showing a significant increase in the percentage of normal glomeruli in the agalsidase α phase III clinical trial^[24]; a significant decrease in the inclusion score in the kidney microvascular capillary endothelial cells in the agalsidase β phase III clinical trial, with a complete clearance of the endothelial, mesangial and distal convoluted tubule/collecting duct cells after 54 months of the extension study^[22,34]; a significantly greater reduction in the mean number of Gb3 inclusion per kidney interstitial capillary than in the placebo group in the migalastat phase III clinical trial^[66].

Nonetheless, clearance of inclusions in glomerular podocytes is much more difficult and time-dependent. The largest published study showed that, after a mean of almost 10 years, there was a significant decrease in the Gb3 inclusions in the podocytes both in the so-called “lower fixed dose group” (0.2 mg/Kg/EOW for the entire follow-up) and in the “higher dose group” (doses higher than 0.2 mg/Kg/EOW for, at least, part of the follow-up period), but with a significant correlation between the cumulative dose of ERT received and the clearance of podocyte Gb3; however, GFR only had a significant decrease in the “higher dose group” and remained stable in the “lower fixed dose group”^[193].

Nevertheless, the importance of the finding of persistent podocyte Gb3 inclusions remains controversial, because its role as a surrogate biomarker for the progression of renal dysfunction (GFR) in patients treated with ERT is not well established. Notwithstanding the significant correlation between the decrease in urinary albuminuria and the decrease in podocyte Gb3 inclusion score in a long-term evaluation of histological outcomes, in the same cohort, GFR remained stable in all patients, regardless of the decrease in podocyte Gb3 inclusions^[18]. Similarly, in the 54-month extension study of the agalsidase β phase III clinical trial, despite persistent inclusion in podocytes in the six evaluated patients, only in one patient, there was a progressive decline in GFR^[34]. Moreover, as mentioned above, even in untreated patients, there is no correlation between semi-quantitative scoring systems for intracellular Gb3 inclusions and GFR^[9,15,17,191] and there is evidence of heavy podocyte inclusions in patients with late-onset variants, despite the small risk of progressive nephropathy^[194,195].

CENTRAL NERVOUS SYSTEM BIOMARKERS

Research for biomarkers of central nervous system (CNS) in FD has been challenging due to the limited knowledge about the physiopathology of CNS involvement in FD. No valuable serum biomarkers exist for the early detection, risk stratification, or monitoring of cerebrovascular disease progression^[196]. There is a weak correlation between serum cystatin-C and CNS pathology in males^[92]. In females, plasma lyso-GB3 correlates with WML severity^[41].

Magnetic resonance imaging

Currently, brain MRI is the most useful tool for evaluating CNS in FD, with several sequences studied as biomarkers of its involvement.

White matter lesions

WML in the form of single, multiple, or confluent hyperintensities in T2-weighted MRI are the most commonly reported image markers of neurovascular involvement in FD patients (present in about 2/3 of the patients, despite the absence of overt clinical signs of cerebral disease)^[197,198]. The localization pattern is

typical of a small vessel disease and is similar to the age-related WML^[199]. Rare in children with FD, the presence and load of WML increase with age, being both genders equally affected^[200]. WML load in FD could be modulated by classical and genetic vascular risk factors and the presence of other organ injury, such as cardiomyopathy or nephropathy^[201]; however, a recent meta-analysis only found a significant correlation between the load of WML and the risk of stroke, but not with the other organs involvement^[202]. The effect of long-term ERT in WML remains controversial, with few studies showing the progression of WML during ERT, but a single study with agalsidase β showed a significantly higher proportion of patients with stabilization or decrease in WML at follow-up in the ERT group in comparison with the placebo group^[203-205].

Diffusion tensor imaging and perivascular spaces

Diffusion tensor imaging (DTI) is able to detect early white matter abnormalities and could be a potential marker of disease progression and treatment response, but it needs to be validated in clinical practice; in FD, DTI showed widespread areas of microstructural white matter disruption in Fabry disease (extending beyond WML seen on conventional MRI), with strong correlations with cognition, clinical disease severity, and plasma lyso-Gb3^[206-208]. An increase in perivascular spaces, mainly in the basal ganglia, was also demonstrated in FD, suggesting that impaired interstitial fluid drainage might be a mechanism of white matter injury in FD^[209].

Basilar artery

Significant enlargement (dolichoectasia) and tortuosity of the intracranial arteries, in particular the basilar artery, are frequently reported in FD patients and distinguish them from controls^[210,211]. Furthermore, vertebrobasilar dolichoectasia could be an early marker of neurovascular involvement, being present in 56% of men and 35% of women and identified even in the absence of WML^[212].

Pulvinar sign and hippocampus atrophy

Increased signal intensity in the pulvinar region on T1-weighted MRI scans (the pulvinar sign) has been described in patients with FD^[213]. Although characteristic, it is not pathognomonic of this disease. It is frequently found in male patients and usually affects both thalamus, although unilateral presentation has been reported. It seems to be present in less than 20% of FD and associated with cardiac and renal dysfunction, but not stroke^[214].

Hippocampus atrophy is another CNS image surrogate reported in FD patients (mainly in males) and not associated with ischemic signs, probably reflecting neuronal direct involvement^[215]. Moreover, a significant decline in hippocampus volumetry is observed over time and does not correlate with increased WML load or cerebrovascular events^[216].

Transcranial Doppler

Transcranial Doppler could also detect abnormalities in brain arteries typical of small vessel disease and abnormal cerebral autoregulation that may be predictive of future neurovascular events in patients with FD^[217]. Of note, a study with functional transcranial Doppler revealed cortical vascular dysfunction in the territory of the posterior circulation in asymptomatic patients^[196].

SUMMARIZING: WHAT SHOULD WE USE IN THE CLINICAL PRACTICE?

In clinical practice, the aforementioned biomarkers should be used for diagnostic purposes, in the preclinical and clinical evaluation, and to assess treatment response. [Figure 2](#) summarizes the usefulness of the described biomarkers in clinical practice according to these different objectives.

	Diagnosis	Clinical monitoring			Treatment
		Pre-clinical	Early	Late	
Lyso-Gb3	✓*				?
Troponin			----->		✓
NT-proBNP			----->		✓
TDI		----->			✓
Speckle-tracking		----->			✓
LGE	✓**			----->	✓***
T1 mapping	✓**	----->			✓
eGFR			----->		✓
Cystatin C			----->		?
Albuminuria			----->		✓***
Brain MRI		----->			?

Figure 2. What biomarkers should we use in clinical practice? *not useful in female patients with late-onset phenotype; **characteristic pattern in FD; ***to predict treatment response; Lyso-Gb3: Globotriaosylsphingosine; TDI: tissue Doppler imaging; LGE: late gadolinium enhancement; eGFR: estimated GFR; MRI: magnetic resonance imaging.

SEARCHING FOR NEW BIOMARKERS

Searching for new biomarkers in Fabry disease is paramount, as no proper or well-established plasma or urinary biomarkers are available in clinical practice to aid the diagnosis, early detection of major organ involvement, and monitoring and evaluation of treatment response. However, biomarker discovery remains a very challenging task due to the complexity of the samples (body fluids or tissues) and the wide dynamic range of molecule concentrations in a heterogeneous disease.

There are two main approaches to discovering new plasma/urine biomarkers in FD: “angling”, a one-by-one approach, which involves the study of candidate biomarkers (for example, tubular proteins or inflammatory mediators) where laboratory studies have suggested a pathological link or with proven value in a similar pathological model; “trawling”, based on “omics” medicine, where biological fluid/tissue is screened for disease-associated molecules (for example, proteins or metabolites) using an array of technologies, predominantly based on mass spectrometry.

One-by-one approach has been widely used and several biomarkers with clear pathological correlation have been found. Moreover, the interpretation of the data and correlation with the clinical variables/disease heterogeneity is easier with this approach. However, most of the serum and urine biomarker studies performed to date seem to have converged on a set of proteins and metabolites that are repeatedly identified in many studies and that represent only a small fraction of the entire proteome/metabolome, so the added value of the one-by-one approach in deepening knowledge about the disease pathophysiology is limited^[218].

In contrast, omics-based applications use technological resources to further expand our knowledge of the complexities of human disease. However, if reliable and useful inferences with potential for translation into clinical practice are to be achieved, omics techniques require understanding inherent biological variables, rigorous methodology, and analytical chemistry tools, the use of instrumentation that ensures high data quality, and consistent and transparent analysis of the generated data^[219]. In proteomics and metabolomics,

Table 5. Experimental biomarkers in Fabry disease

Biomarkers	Comment	References
Inflammatory	C-reactive protein was studied with conflicting results; IL-6 increases in patients with FD cardiomyopathy and decreases during ERT; IL-18 increases even in early stages of FD cardiomyopathy and decreases with ERT; Myeloperoxidase and chitotriosidase only increase in males	[232-235,236,237,238]
Coagulation and endothelial dysfunction	Conflicting results, with minimal and inconsistent abnormalities in markers of platelet and coagulation activation; Homocysteine increases in FD and one study reported an increase in all the patients presenting cerebrovascular disease; Nitric oxide metabolism biomarkers seem altered in FD and correlate with cardiomyopathy severity	[239,234,240-243]
Vasculopathy	Sphingosine-1-phosphate promotes vascular smooth muscle cell proliferation and correlates with carotid artery intima-media thickness and LV mass index	[244]
Myocardial fibrosis	Few studies show an increase in biomarkers of collagen type 1 synthesis and a decrease in matrix metalloproteinases, correlating with LV mass and FD cardiomyopathy progression; Galectin-3 presents a significant increase, even in patients without signs of cardiac involvement, with a significant correlation with LV mass and GFR	[144,245-247]
Urinary microscopy	May be a useful tool for the non-invasive assessment of disease progression; it presents some limitations: its diagnostic value is not well established in patients with late-onset phenotypes, most of the findings are not pathognomonic of FD, and its prognostic value needs further evidence	[248]
Podocyturia	Podocyte counting in urine sediments is time-consuming and technically challenging to obtain reliable data (reading is observer-dependent). Inconsistent correlations with albuminuria and GFR are found; thus, added value against albuminuria, in terms of diagnostic accuracy, nephropathy prognosis, and response to ERT, has not been established	[249-254]
Tubular injury	N-acetyl- β -glucosaminidase presents an inverse correlation with estimated GFR stronger than the one between estimated GFR and albuminuria and is a good predictor of nephropathy progression. Uromodulin and bikunin were studied in small cohorts, with conflicting results	[255-258]
Plasma metabolomics	Analogs/isoforms of Gb3 and lyso-Gb3 increase in plasma (to a lesser extent than plasma lyso-Gb3); all of them are significantly more elevated in male patients (compared with female patients) and most of them show a significant decrease during ERT	[47,221,222]
Urinary metabolomics	Increased excretion of lyso-Gb3, Gb3, and galabiosylceramide isoforms/analogues (all but one of the lyso-Gb3 analogues has relative concentrations that are higher than lyso-Gb3), with a more prominent increase in male patients and a significant decrease during ERT	[47,223-225]
Plasma proteomics	Conflicting results, with different protein profiles found	[259-261]
Urinary proteomics	The most consistent alterations found are the up-regulation of prostaglandin H2 D-isomerase and prosaposin (the latter is also observed in pediatric, pre-symptomatic patients); both are known to play roles in processes that might be involved in FD pathophysiology	[226-231]
MicroRNAs	Few microRNAs present very good diagnostic accuracy in distinguishing between classical, late-onset phenotypes, and other forms of cardiomyopathy; some correlations with GFR are found	[46,262,263]
Circular RNAs	Few circular RNAs are differentially expressed in FD patients and circulating levels are related to the phenotype and disease severity	[264]

IL: Interleukin; ERT: enzyme replacement therapy; FD: Fabry disease; LV: left ventricle; GFR: glomerular filtration rate.

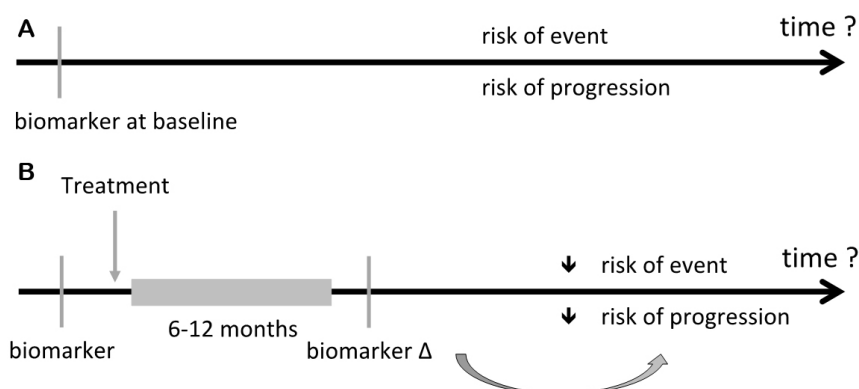


Figure 3. Proposed study design to evaluate biomarkers. (A) biomarkers to assess patient prognosis; (B) biomarkers to assess response to treatment.

there are several challenges and limitations that need to be overcome to keep pace with advancements and ultimately realize clinical applicability. These hurdles include the complexity of proteome (a large number of structural and biochemical differences of proteins), a very wide range of protein concentrations, complex sample preparation and data analysis (most reported biomarkers remain unidentified), and limited sensitivity. Additionally, the capacity for quantitative measurements is not yet at the level required for routine diagnostics in a clinical setting. Furthermore, it remains unclear how clinicians will use the sensitive data since even small changes in physiology, such as food ingestion or going up a flight of stairs, can have a significant impact on the metabolome^[220].

Despite these limitations, plasma and urinary metabolomics showed that Gb3, lyso-Gb3, and galabiosylceramide isoforms/analogues were elevated in FD patients^[47,221-225]. However, these candidate biomarkers still require validation that they overcome the lyso-Gb3 limitations. This validation includes the evaluation of late-onset females and patients with variants of unknown significance and correlation with clinical manifestations and treatment responses. Thus, properly designed longitudinal studies are warranted.

The proteomic approach has also been applied in the search for biomarkers of FD in plasma, peripheral blood mononuclear cells, and urine. Urinary proteome is the most extensively studied in FD, with a few studies with up to 66 patients reporting several proteins with altered expression^[226-231]. A panel of biomarkers including 40 proteins were able to show proteins that are related to early/preclinical phase of FD, to monitoring kidney injury, and to heart involvement; however, the actual prognostic significance of these panels was not clearly depicted and should be evaluated further^[231].

Designing studies to identify new biomarkers is also challenging and the design should be tailored to the specific objective of the study/biomarker: diagnostic purposes, identify preclinical involvement, clarify the prognosis, or assess the response to the treatment. For example, to assess the prognosis and the response to DST, a complex design is warranted [Figure 3]. To identify prognostic biomarkers, several premises should be taken into account in the study design, including longitudinal and prospective design, follow-up duration, and a clear definition of adverse events and disease progression. In studies assessing the response of a specific biomarker to DST, beyond the described aspect, it is paramount to clarify the definitions of treatment response and failure. Defining the duration of a biomarkers study is difficult, because FD is a slowly progressive disorder with a wide spectrum of severity/phenotypes and a low event rate.

Several protein and lipid experimental biomarkers are under investigation in Fabry disease [Table 5], mainly related to inflammation, endothelial dysfunction, cardiac fibrosis, glomerulosclerosis, and tubulointerstitial fibrosis. Furthermore, there are also few reports on proteomic and metabolomics analysis.

CONCLUSION

The identification of biomarkers for identification of preclinical involvement, prognostic evaluation, and response to treatment is an urgent need in FD. The available biomarkers of total disease burden, such as plasma lyso-Gb3, have several limitations in prognostic evaluation and monitoring treatment response.

Thus, accurate longitudinal studies are needed to identify new biomarkers and their prognostic value. Furthermore, FD has a very complex physiopathology, and certainly, no single biomarker is able to characterize all the pathways involved, so composite scores of clinical and laboratory variables should be the only method to assess each patient's prognosis and response to treatment.

DECLARATIONS

Authors contributions

The author contributed solely to the article.

Availability of data and materials

My PhD thesis was related to biomarkers in AFD and this similarity is due to my own work.

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Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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