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Review article

An expert consensus on the recommendations for the use of biomarkers in Fabry disease

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ABSTRACT

Fabry disease is an X-linked lysosomal storage disorder caused by the accumulation of glycosphingolipids in various tissues and body fluids, leading to progressive organ damage and life-threatening complications. Phenotypic classification is based on disease progression and severity and can be used to predict outcomes. Patients with a classic Fabry phenotype have little to no residual α-Gal A activity and have widespread organ involvement, whereas patients with a later-onset phenotype have residual α-Gal A activity and disease progression can be limited to a single organ, often the heart. Diagnosis and monitoring of patients with Fabry disease should therefore be individualized, and biomarkers are available to support with this. Disease-specific biomarkers are useful in the diagnosis of Fabry disease; non-disease-specific biomarkers may be useful to assess organ damage. For most biomarkers it can be challenging to prove they translate to differences in the risk of clinical events associated with Fabry disease. Therefore, careful monitoring of treatment outcomes and collection of prospective data in patients are needed. As we deepen our understanding of Fabry disease, it is important to regularly re-evaluate and appraise published evidence relating to biomarkers. In this article, we present the results of a literature review of evidence published between February 2017 and July 2020 on the impact of disease-specific treatment on biomarkers and provide an expert consensus on clinical recommendations for the use of those biomarkers.

Contents

Abbreviations: α-Gal A, α-galactosidase A; ACE, angiotensin converting enzyme; ADA, anti-drug antibody; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CMB, cerebral microbleeds; CMR, cardiac magnetic resonance; CMRi, CMR imaging; CNS, central nervous system; DBS, dried blood spot; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; EOD, every other day; EOW, every other week; ERT, enzyme replacement therapy; EU, European Union; FASTEX, FAbry STabilization indEX; FD, Fabry disease; GFR, glomerular filtration rate; GL3, globotriaosylceramide; HCM, hypertrophic cardiomyopathy; hs-cTnT, high-sensitivity troponin-T; IAR, infusion-associated reaction; IgE, immunoglobulin E; IgG, immunoglobulin G; lyso-GL3, globotriaosylsphingosine; LVH, left ventricular hypertrophy; LVM, left ventricular mass; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PVS, perivascular space; QoL, quality of life; SVD, small vessel disease; UPCR, urine protein/creatinine ratio; VEGF, vascular endothelial growth factor; VUS, variant of unknown significance; WMH, white matter hyperintensities.

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1. Introduction

Fabry disease (FD) is a rare, X-linked lysosomal storage disorder caused by pathogenic variants in the *GLA* gene encoding the enzyme α-galactosidase A (α-Gal A) [1]. Deficient or absent α-Gal A and the subsequent accumulation of glycosphingolipids such as globotriaosylceramide (GL3) and globotriaosylsphingosine (lyso-GL3) in various tissues and cells cause progressive damage to affected organs, life-threatening complications, and increased risk of premature death [2]. FD can be classified into two phenotypes: classic and later-onset. Males with classic FD have severely reduced or absent α-Gal activity and generally experience signs and symptoms from early childhood onwards, including progressive cardiac, cerebral, and renal involvement [2,3]. Diagnosis of males with classic FD is confirmed if the activity of α-Gal is not detectable or is <1% of the expected value [2]. Lyso-GL3 levels in individuals with classic FD are usually higher than in patients with later-onset FD, and these levels are higher in males than females with classic FD. Individuals with a later-onset phenotype have higher residual α-Gal activity and can be more challenging to diagnose because disease manifestations may be limited to a single organ and there may be a lack of early clinical symptoms including neuropathic pain, sweating abnormalities, and angiokeratoma [2,3]. *GLA* gene analysis is required for a definitive diagnosis in females and is advisable in all patients to assist in prediction of the phenotype. Female carriers of pathogenic *GLA* variants display variable clinical penetrance, which is in part due to the process of lyonization [4].

Enzyme replacement therapy (ERT) with human α-Gal has been the mainstay of FD-specific treatment to delay or prevent progressive organ damage and improve disease symptoms. There are two forms of intravenous ERT available: agalsidase alfa (Replagal®, Takeda) and agalsidase beta (Fabrazyme®, Sanofi) [5], both approved in the European Union (EU) in 2001. Oral chaperone therapy, migalastat (Galafold®, Amicus), was approved for the treatment of patients with FD in the EU in 2016 [6] and in the US in 2018 [7]. Migalastat reversibly binds to the active site and stabilizes mutant forms of α-Gal, thereby promoting trafficking to lysosomes and substrate catabolism. Chaperone therapy is only approved and suitable for patients carrying amenable *GLA* variants, which is defined as α-Gal A activity in the presence of 10 μmol/L migalastat that is ≥1.2-fold over baseline with an absolute increase of ≥3.0% wild-type α-Gal A activity [6,8]. Owing to the clinical heterogeneity of FD, the effect of disease-specific therapies varies according to disease severity, point of treatment initiation, the presence or absence of anti-drug antibodies in males, and patient adherence [9]. Therefore, therapeutic goals need to be individualized [10,11].

Early diagnosis of FD is essential for the timely initiation of FD-specific treatment [2,12], to delay or stop disease progression, reduce disease-associated morbidity, prolong survival, and improve patient quality of life (QoL) [13]. The identification of reliable and validated biomarkers for diagnosis and monitoring of disease progression and organ-specific pathology is crucial to improving patient outcomes because they can inform decisions on when to initiate or switch therapy and help in predicting events that may require organ-specific therapy [14,15].

The National Institutes of Health Biomarkers Definitions Working Group defines a biomarker 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” [16]. Broader definitions of biomarkers include imaging studies to diagnose and assess the severity of a disease and determine prognosis [17]. Characteristics of an ideal biomarker depend on its intended use (Fig. 1). Prognostic biomarkers assess the likely course of a disease with or without therapy and predictive biomarkers identify individuals who are likely to respond to a given specific or targeted therapy. Pharmacodynamic biomarkers measure the effect of a drug on the disease state itself [18]. The complexity of lysosomal storage disorders, including FD, and their multi-system impacts mean that multiple biomarkers may be valuable in assessing disease course and response to therapy (Fig. 2).

GL3 and lyso-GL3 have been used extensively as biomarkers of FD for diagnostic purposes and to assess treatment outcomes in clinical studies; however, their use in monitoring disease progression and treatment response outside of clinical trials of ERT has only been employed in a small number of longitudinal studies [19,20]. Kidney biopsies have shown increasing GL3 accumulation with age [21], and clinical studies have shown GL3 clearing of multiple kidney cell types in patients receiving ERT or chaperone therapy [22–24]. However, some

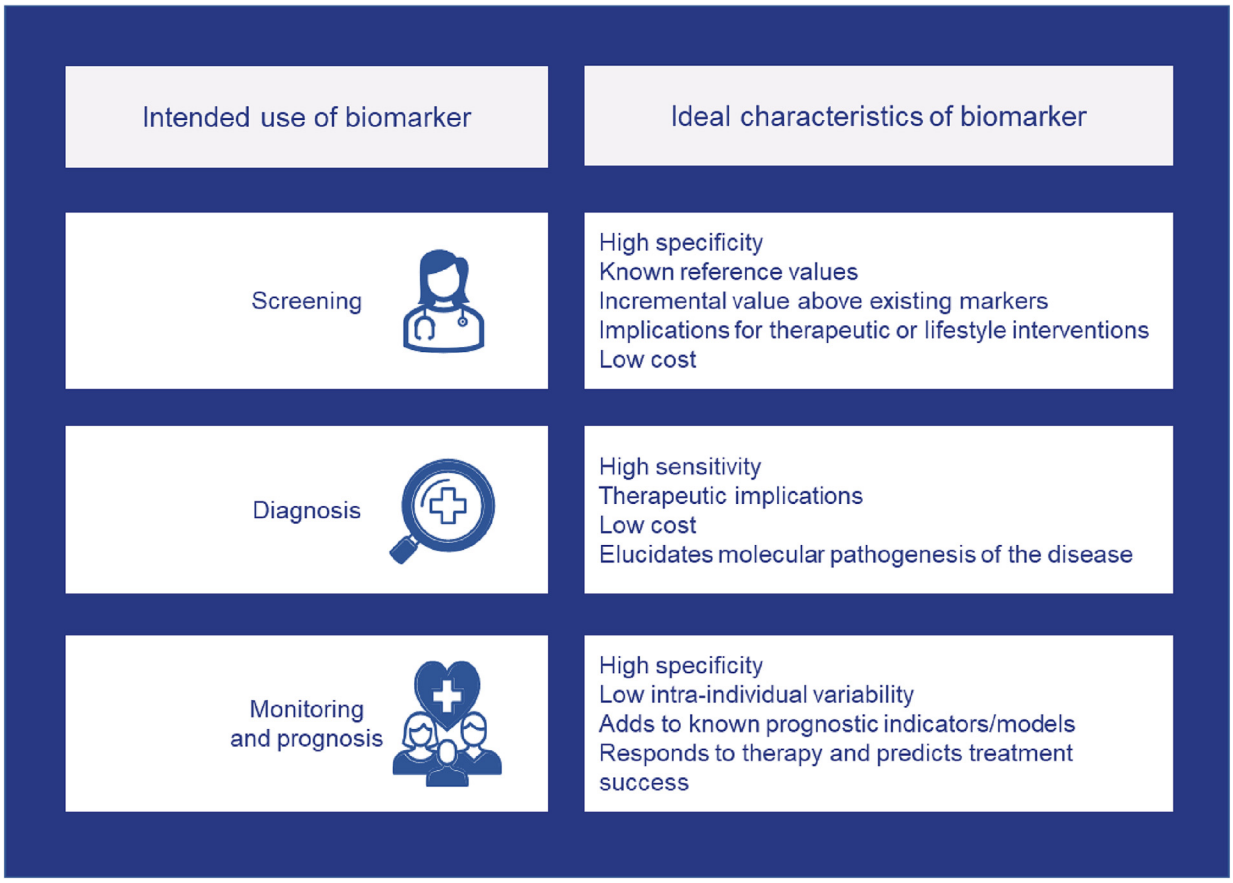

Fig. 1. Ideal characteristics of a biomarker for disease screening, diagnosis, monitoring, and prognosis.

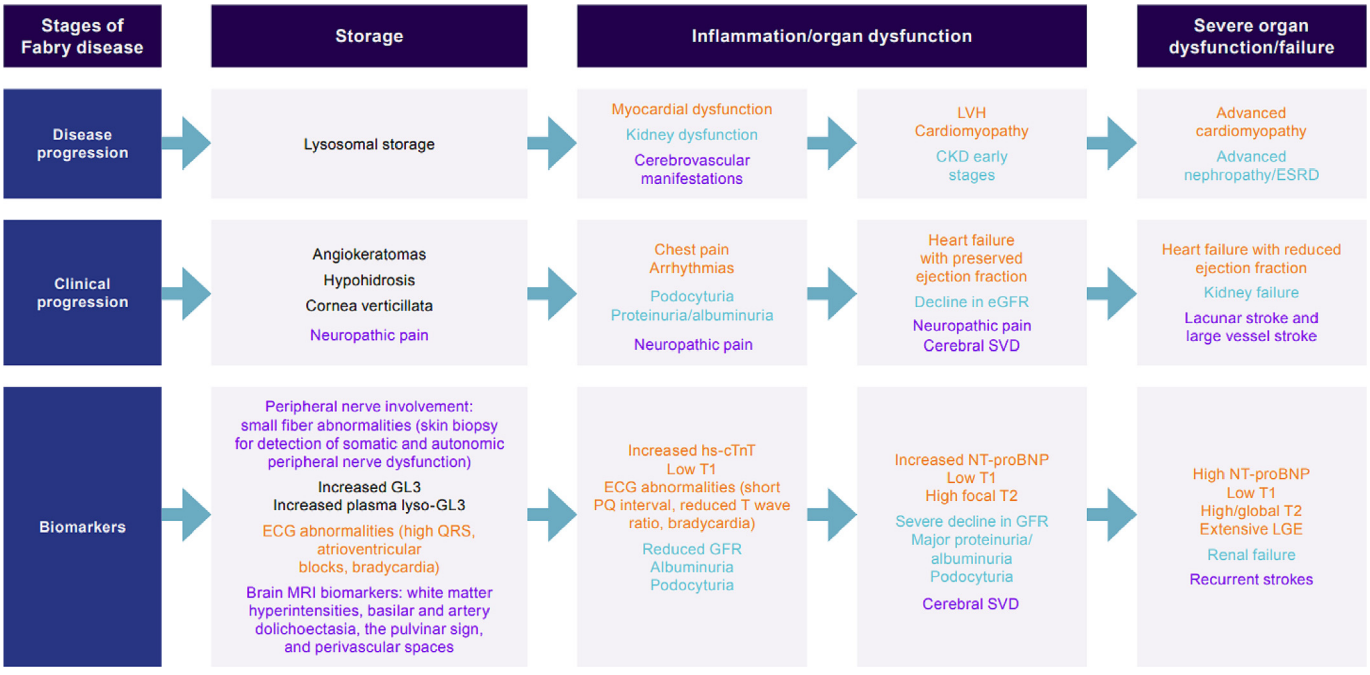

Fig. 2. Correlation of biomarkers in Fabry disease with disease severity.
Cardiovascular manifestations in orange text, renal manifestations in turquoise text, cerebrovascular manifestations in purple text, and other Fabry disease symptoms/manifestations in black text.
CKD, chronic kidney disease; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; hs-cTnT, high-sensitivity troponin T; LGE, late-gadolinium enhancement; LVH, left ventricular hypertrophy; lyso-GL3, globotriaosylsphingosine; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SVD, small vessel disease.

patients show disease progression and persistently elevated levels of plasma GL3 and lyso-GL3 despite ERT. This may be due to the phenotypic heterogeneity of FD, type or dose of ERT, point of treatment initiation, or the inhibitory effect of anti-agalsidase antibodies [3,25–29]. Other biomarkers relating to changes in organ architecture or function have been identified, but few have been studied to evaluate the response to ERT [14]. Furthermore, there is little evidence relating to the effect of chaperone therapy on disease- and organ-specific biomarkers [30].

As our understanding of FD improves and more treatment options become available, it is important to regularly re-evaluate and appraise published evidence relating to disease-specific biomarkers, treatment outcomes, and therapeutic goals for patients with FD. At present, the interpretation of published evidence of disease-specific therapies and biomarkers of FD is hindered by the relative lack of robust randomized clinical trials and the heterogeneity of patients included in different studies [31].

2. Objectives

In 2019, a European panel of experts collaborated to develop a set of organ-specific therapeutic goals for FD based on outcomes identified in a structured literature review of published evidence from adult male, adult female, and pediatric patients, and consensus opinion [13,32–34]. We now present the results of an updated structured literature review that explored the effect of FD-specific treatments on FD- and organ-specific biomarkers in male and female patients with classic and later-onset disease. The objective of this article is to provide clinical recommendations for the use of FD- and organ-specific biomarkers in various tissues and body fluids, including blood and urine, as indicators of disease severity, progression, and response to FD-specific therapies, based on that updated structured literature review.

3. Methods

The full methodology for the structured literature searches that were performed for the first analysis have been described previously, together with the expert consensus recommendations [13]. In this updated analysis, an extensive literature search, for articles published between February 2017 and July 2020 (inclusive), was conducted using the EMBASE and PubMed databases that included outcome data for approved dose regimens of agalsidase alfa 0.2 mg/kg every other week (EOW), agalsidase beta 1.0 mg/kg EOW, or oral migalastat 123 mg once every other day (EOD). Evidence from studies of adult male, female, or mixed-sex (male and female) patient populations was graded on the basis of study type: randomized clinical trials (grade 1 evidence), comparative and switch studies (grade 1), prospective and retrospective observational studies (grades 2 and 3 respectively), case series (grade 4), and case reports (grade 5) [35]. The findings from clinical trial and observational studies in this updated analysis are listed in **Supplementary Table 1**. Specific note has been made of altered dose regimens due to the temporary shortage of agalsidase beta to examine the efficacy of reduced-dose ERT [36]. Since the cut-off date for the original literature search, several studies of interest have been published. Although these were not incorporated in the original analysis, they warrant inclusion for discussion in this article.

Outcomes that were selected for analysis included plasma and urine GL3 and lyso-GL3 levels, biomarkers associated with kidney, heart, and central nervous system (CNS) function, and immunogenicity or seroconversion. GL3 and lyso-GL3 levels were described as 'normalized' if they were higher than reference values at baseline (pre-treatment) and decreased to within reference value ranges during treatment, and if they were described as normalized in the publication; note that the reference values varied among studies. Biomarkers associated with the peripheral nervous system were excluded from this report due to limited data identified during the literature search. Kidney, skin, and heart biopsy outcomes were excluded because the procedure is invasive, and this review focuses on non-invasive biomarkers of FD.

To our knowledge, this is the first time that this most recent evidence on FD biomarkers has been comprehensively reviewed. Most publications focused on the effect of disease-specific therapies on plasma lyso-GL3, highlighting the growing interest in the utility of this biomarker in early diagnosis and therapeutic monitoring. Our findings also reflect the need to identify disease- and organ-specific biomarkers that reflect early pathological changes associated with FD, before the manifestation of significant organ damage.

4. The impact of treatment outcomes on biomarkers of Fabry disease

A total of 119 publications were identified for inclusion. The key recommendations for the practical application of FD- and organ-specific biomarkers in clinical practice are provided in Table 1.

4.1. GL3 and lyso-GL3

FD results in the accumulation of glycosphingolipids, GL3 and its deacylated form, lyso-GL3. Therefore, these biomarkers have been studied extensively for the diagnosis, treatment, and monitoring of FD, particularly in the context of clinical studies [14]. Evidence from the literature, as well as this analysis, suggests that the measurement of lyso-GL3 in plasma and/or in dried blood spots (DBS) [37,38] is widely used in the biochemical confirmation of the diagnosis of FD, assessment of the phenotype and monitoring the response to ERT, as well as response following specific treatment modifications, including switching therapies [27,39].

4.1.1. Plasma GL3

A reduction in the levels of plasma GL3 with agalsidase beta was observed in one study [23] identified in the timeframe of this literature search, spanning from February 2017 to July 2020. Studies with data on the effects of agalsidase alfa or migalastat were not identified, but since this review, additional studies have been reported. Many centers have replaced measurements of plasma GL3 with lyso-GL3 as the fluctuation of lyso-GL3 seems to correlate better with disease activity [40].

4.1.2. Plasma, serum, and dried blood spot lyso-GL3

Lyso-GL3 can be measured directly in plasma and/or in DBS so the methodology applied should be considered when comparing lyso-GL3 outcomes [37]. Nowak *et al.* showed a good correlation between lyso-GL3 concentrations in DBS and sera [27,38]. The lyso-GL3 level in sera can be estimated from the DBS concentration by multiplying the DBS value by 1.5. Another group showed good correlation between lyso-GL3 concentrations in plasma and DBS [37]. In a recent study by Maruyama *et al.*, plasma lyso-GL3 was found to be an effective selective screening marker for classic and later-onset male and female patients with FD, thus identifying unrecognized FD cases [41]. Moreover, the demonstration of normal lyso-GL3 levels in plasma and/or DBS is crucial for the phenotypic characterization of individuals with *GLA* variants of unknown significance (VUS), as also demonstrated by newborn screening studies in which lyso-GL3 was used as a second tier test [42,43]. However, it should be noted that plasma lyso-GL3 levels may also be elevated in patients with Gaucher disease [37,44].

Generally, plasma lyso-GL3 levels decrease during the first 3 months of ERT [45]. It is still unclear whether a reduction in lyso-GL3 correlates with a reduction in clinical events in patients with FD [46]. Overall, studies demonstrate that both agalsidase alfa and agalsidase beta significantly reduce plasma lyso-GL3 in male patients with a classic FD phenotype who have very high baseline levels. Conversely, reduction of lyso-GL3 levels in males with a later-onset phenotype and in all females is likely to be minimal because baseline lyso-GL3 levels are only slightly elevated in this cohort of patients [27,30,47–50].

Table 1
Key recommendations for the practical application of FD- and organ-specific biomarkers in clinical practice.

Biomarker	Application	Recommendations
Plasma GL3	• Minor use in clinical practice	• Not recommended, replaced by lyso-GL3
Plasma and/or DBS lyso-GL3	• In the diagnosis and follow-up of patients with classic FD, regardless of age or sex of the patient • Assess in male patients with classic FD for monitoring pharmacodynamic response to the treatment	• Assists in the stratification of phenotypes associated with mutations [27,39] • All patients with classic FD should be monitored when treatment is started and 6–12 months thereafter. If treatment is switched from ERT to migalastat, monitor every 6 months. An inconsistent reduction during ERT may indicate the formation of neutralizing antibodies [27,39,136] • Limited evidence of benefit following change in ERT type in terms of predicting clinical events
Urinary lyso-GL3	• Not used in clinical practice	• Not recommended [152]
Troponin, hs-cTnT and NT-proBNP	• To be performed systematically at diagnosis and in follow-up of any patient	• Assess in all patients when treatment is started and at least annually thereafter [89] • As per general therapy – lack of evidence of a specific role in FD
Albuminuria	• In the diagnosis and follow-up of patients	• Assess in all patients when treatment is started and at least annually thereafter [95] • Assess need for antiproteinuric therapy and risk stratification
Serum creatinine	• In the diagnosis and follow-up of patients	• In all patients when treatment is started and at least annually thereafter (depending on CKD stage) • Monitor kidney function [70]
Podocyturia	• Not routinely used because only available in specialized centers	• Not recommended
eGFR (CKD-EPI equation)	• In the diagnosis and follow-up of patients	• In all patients when treatment is started and at least annually thereafter (depending on CKD stage) • Monitor kidney function [70]
Anti-drug antibodies and inhibition of α-Gal A activity	• In the follow-up of male patients	• In all male patients when treatment is started and at least annually thereafter [113]
ECG	• Diagnosis and monitoring	• Annually and as clinically indicated [2]
Cardiac MRI	• In the diagnosis and follow-up of patients	• MRI with gadolinium: Regularly (>2 year interval) or when disease clinical progression is evident [2] • MRI with T1 mapping: Used as an investigational tool [2]
Brain MRI	• In the diagnosis and follow-up of patients	• Regularly (every 3 years) or when clinically needed [2]

CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease - Epidemiology Collaboration; DBS, dried blood spot; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; FD, Fabry disease; GL3, globotriaosylceramide; hs-cTnT, high-sensitivity troponin T; lyso-GL3, globotriaosylsphingosine; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Evidence identified in this literature search suggests that ERT switch from agalsidase alfa to agalsidase beta was associated with further reductions in plasma lyso-GL3 [25,51]. Evidence of the effect of migalastat on plasma lyso-GL3 identified in this literature review is still unclear. Switching treatment from ERT to migalastat sometimes results in a novel increase of lyso-GL3 levels in plasma, particularly in patients with very low residual enzyme activity and low increase of enzymatic activity after incubation with migalastat [52,53].

Findings from this analysis and the wider literature do not indicate a clear correlation between lyso-GL3, measures of FD progression and chaperone therapy responses. *Post hoc* analysis of treatment-naïve and ERT-experienced patients from the FACETS and ATTRACT studies (with subsequent open-label extensions) found no significant correlations between changes in lyso-GL3 and changes in other FD parameters such as left ventricular mass (LVM) index, estimated glomerular filtration rate (eGFR), or manifestations such as pain. However, a longitudinal correlation was identified between lyso-GL3 and worst pain in 24 h in the overall treatment group ($N = 97$), and between lyso-GL3 and LVM index in patients aged >40 years [30]. Lyso-GL3 may not always be a suitable biomarker for monitoring treatment response in patients with FD because the reversal of dysregulated cellular mechanisms in FD may not be possible with ERT after a certain point [54], at least in podocytes. In addition, neither baseline lyso-GL3 nor the rate of change in lyso-GL3 during treatment predicted the occurrence of Fabry-associated clinical events in all patients or those receiving migalastat for ≥24 months [30].

In addition to its usefulness as a diagnostic biomarker of FD, plasma lyso-GL3 may be useful in the assessment of disease severity, as it has been shown to correlate with Mainz Severity Score Index and DS3 scores [48,55,56]. However, although lyso-GL3 has been used extensively to evaluate treatment outcomes with respect to clinical studies of ERT, its utility in assessing clinical response outside this setting has not been validated, and few longitudinal studies have investigated lyso-GL3 for FD progression and clinical response to therapy for the guidance of treatment decisions [19,20]. Evidence relating to the prognostic value of lyso-GL3 is mixed. An explanation for this could be the different statistical method used in these studies, for example, some studies adjusted for factors that are closely correlated with lyso-GL3 e.g., age, sex, and/or phenotype or there may be other factors which are more closely associated with prognosis. A study by Arends *et al.* adjusted the hazard ratio for age at the start of ERT, sex, and phenotype and showed that neither the lyso-GL3 concentration at baseline, lyso-GL3 concentration during treatment, absolute decrease of lyso-GL3, nor the relative decrease of lyso-GL3 predicted the risk of clinical events in patients receiving ERT [19]. A study by Nowak *et al.* adjusted for male sex, age, and classic phenotype and found higher baseline serum lyso-GL3 levels were associated with significant composite clinical events such as new onset of stroke, atrial fibrillation, kidney failure requiring kidney replacement therapy, and death. This finding suggests that lyso-GL3 may be a useful biomarker for risk stratification in patients with FD [57]. In line with this, another study found that higher plasma lyso-GL3 levels were associated with increased prevalence of cardiomyopathy, nephropathy, and cerebrovascular disease, but that levels of lyso-GL3 remained unaltered over 6–18 months of repeat testing, and were independent of sex, *GLA* mutation, or treatment status [58]. Furthermore, a study by Rombach *et al.* identified the importance of plasma lyso-GL3 as a screening tool for female patients with left ventricular hypertrophy (LVH) but found no apparent correlation between plasma lyso-GL3 concentrations and kidney failure, microalbuminuria, and proteinuria in their female cohort [48]. In contrast, a study by Nowak *et al.* found serum lyso-GL3 to be independently associated with Fabry nephropathy, cardiomyopathy, and history of stroke or transient ischemic attack [27].

In summary, lyso-GL3 is an easy-to-measure circulating compound in patients with FD, with utility in diagnosis and assessment of pharmacodynamic effect of treatments. The evidence on lyso-GL3 as a measure

of clinical severity and as a prognostic indicator is mixed, as documented in the studies cited above. Further exploration of the correlation between lyso-GL3 and the degree of target organ involvement is warranted. Nonetheless, plasma lyso-GL3 has wider clinical applications than more invasive but definitive assessments of histological accumulation of tissue GL3 that require biopsies of the kidneys or heart and are not part of routine clinical practice.

4.1.3. Urinary GL3

Little evidence on urinary GL3 outcomes was identified in this literature search. One publication reported no significant difference from baseline in 15 male hemizygotic patients treated with agalsidase alfa during a follow-up period of up to 16 years [59]. Regular measurements of urinary GL3 for diagnosis and monitoring are not recommended and further studies are required to investigate the potential use of urinary GL3 in the diagnosis and monitoring of FD.

4.1.4. Urinary lyso-GL3

Two studies reported reductions in urinary lyso-GL3 in patients treated with agalsidase beta, one in a mixed-sex and -phenotype cohort that was switched from agalsidase alfa to agalsidase beta, and the other in male pediatric patients with classic FD [23,60]. Some evidence identified in this literature review suggests that agalsidase beta may reduce urinary lyso-GL3 in male and female patients with classic and later-onset FD, and that treatment switch may be an effective strategy [23,60]. Data on the effects of agalsidase alfa or migalastat were not identified by this literature search.

4.1.5. Summary of GL3 and lyso-GL3

Plasma lyso-GL3, and more recently serum and DBS lyso-GL3, have been established as a diagnostic biomarker in patients with FD and may assist with the stratification of phenotypes associated with FD [27,39]. Given that lyso-GL3 levels are shown to be reduced in response to ERT, particularly within the first 3 months after initiation and in all patients with a classic phenotype and male patients, they may be useful in monitoring FD treatment effect in these groups of patients [27,39]. We recommend measuring lyso-GL3 levels at baseline (before treatment initiation) then every 6–12 months thereafter to evaluate longitudinal changes. However, it is important to note that there is still uncertainty about whether reduction in lyso-GL3 levels correlates with reduced incidence of clinical events, as it may take at least 6 months to observe an improvement in any clinical events in patients with FD and it is not clear if any correlation is specific to modality of therapy [46]. The use of complementary organ-specific biomarkers is recommended in monitoring patients with FD and there is a need to further explore how and whether lyso-GL3 correlates with surrogate disease markers such as LVM index, LVH, eGFR, or clinical events such as stroke, or indices of disease severity.

4.2. Heart biomarkers

Cardiac involvement is the main cause of poor QoL and death in patients with FD [2]. In males with classic FD, involvement begins early in life, progressing sub-clinically before overt symptoms occur and usually manifesting as LVH mimicking hypertrophic cardiomyopathy (HCM), in addition to other multi-systemic symptoms [61,62]. However, a large number of patients have a later-onset FD phenotype that predominantly affects the heart (particularly with cardiac variant p.Asn215Ser) and manifests mostly as LVH or HCM [63–66]. Early diagnosis remains essential to maximizing the benefits of disease-specific therapies, including the improvement of cardiac manifestations. Studies have shown that the benefits of ERT are limited when initiated in patients with advanced disease with considerable LVH or cardiac fibrosis [67–69]. The use of cardiac biomarkers and imaging tools to accurately stage cardiac involvement therefore has important clinical implications.

A comprehensive cardiological work-up should include assessments with electrocardiogram (ECG) and imaging by both echocardiography and cardiac magnetic resonance (CMR) in patients with FD [70]. Subtle electrocardiographic changes precede LVH and may occur early in life [71,72], meaning ECG may also be a useful imaging biomarker to assess cardiac involvement before the onset of HCM [73]. One of the first signs, and sometimes the only sign, of cardiac involvement is shorter PR intervals owing to progressive infiltration of GL3 in the heart. As the disease progresses, conduction time may increase, and ECG may show atrioventricular block [70].

In addition to ECG, CMR imaging (CMRi) in conjunction with biomarker testing may also facilitate the early detection of cardiac involvement in FD. Recent evidence indicates a correlation between plasma lyso-GL3 and LVH [71,74–76]. In addition to GL3 accumulation, inflammation and immune dysfunction are key secondary mechanisms of cardiac damage in FD [77–79]. Preliminary findings suggest a correlation between lyso-GL3, inflammatory, and cardiac remodeling biomarkers and disease progression, indicating a potential role for plasma levels of inflammatory biomarkers in the earlier diagnosis of FD and staging of cardiac disease before significant cardiac damage has occurred [80]. CMRi has become central to the early differential diagnosis and staging of cardiac FD, with CMR studies utilizing T1 and T2 mapping to assess myocardial lipid content and inflammation at different disease stages [81,82]. In the pre-hypertrophic stage of FD low values of T1 correlate with ECG, morphological cardiac changes, and global disease severity as measured by the FAbry STabilization indEX (FASTEX) score [74].

4.2.1. Troponin, high-sensitivity troponin-T (hs-cTnT), B-type natriuretic peptide (BNP), and N-terminal pro-B-type natriuretic peptide (NT-proBNP)

Troponin, hs-cTnT, BNP, and NT-proBNP are important for cardiac disease staging in FD [83–85]. Plasma NT-proBNP is elevated in patients with cardiac manifestations and correlates with symptom class and echocardiographic changes including left atrial size and E/e' (indicating elevated filling pressure) and LVM. Although the highest values are encountered in patients with LVH, diastolic dysfunction, reduced T1 relaxation times on CMRi mapping, and myocardial fibrosis, NT-proBNP concentrations may be raised in patients without echocardiographic evidence of LVH, suggesting that NT-proBNP may be used to assist in decisions on treatment initiation [81,83,86]. Elevated hs-cTnT indicates advanced disease and a poor prognosis [87].

The evidence we identified does not suggest a consistent effect of ERT on hs-cTnT and NT-proBNP in male and female patients with FD, and it is uncertain whether there is any phenotype-specific effect. In one study, 14 patients with normal BNP levels at baseline remained within the same range during treatment with agalsidase alfa, whereas 22 patients with abnormally high BNP levels (≥19.5 pg/mL) had gradually decreasing levels after agalsidase alfa initiation [88]. Another study demonstrated no change in NT-proBNP levels after 1 year of ERT. This study also reported a significant temporal increase in troponin in male and female patients with FD (phenotype not reported) who had been established on agalsidase alfa or agalsidase beta for 1 year [82]. Data on the effects of migalastat on these markers were not identified in the literature search.

Although troponin, hs-cTnT, BNP, and NT-proBNP are organ-specific biomarkers and therefore can be valuable in the follow-up of organ involvement in patients with FD, they are not disease-specific and should not be used to support a diagnosis of FD. Disease-specific biomarkers such as lyso-GL3, which may be elevated in childhood and long before cardiac symptoms manifest, may be useful in predicting a FD diagnosis [43,89]. The combination of hs-cTnT and BNP may be more useful in detecting myocardial fibrosis in patients with HCM. It has been demonstrated that hs-cTnT is a direct marker of ongoing myocardial fibrosis and that BNP is a marker of left ventricular overload partially associated with myocardial fibrosis [90,91]. It is noteworthy that two publications identified elevated troponin in both males and females with FD [82] and

serum vascular endothelial growth factor (VEGF) [92] despite ERT, and one publication did not identify any change in NT-proBNP in newly treated patients or those established on ERT [82], which suggests that cardiac pathology could progress, or that ERT may have been initiated too late in the disease course to mitigate damage to the heart.

4.2.2. Summary of key recommendations for cardiac biomarkers

Cardiac assessments of patients with FD can assist in the staging of cardiac involvement and can therefore influence treatment and monitoring decisions. ECG and cardiac imaging tools may assist with the early identification of cardiac involvement in FD [70], meaning these are important biomarkers to measure in patients with suspected FD. Non-specific cardiac biomarkers of FD can also be used in the diagnosis of patients with FD; however, these are less reliable than other disease-specific biomarkers such as lyso-GL3, which may be elevated in infancy/childhood before the onset of cardiac involvement [89]. Therefore, as noted previously, biomarkers should be used in conjunction to support the diagnosis, treatment, and monitoring outcomes of patients with FD.

4.3. Kidney biomarkers

Nephropathy is a prominent feature of FD and presents with a wide spectrum of severity in male and female patients. Non-invasive biomarkers of kidney injury are found in urine and blood, and the biomarker excretion in urine reflects abnormal nephron function secondary to FD. Most of the publications identified in this structured literature review examined the effect of ERT on kidney outcomes, whereas there were relatively few publications reporting studies that assessed the effect of chaperone therapy on kidney outcomes. This may have been due to the treatment choice based on mutations presented by patients in the studies conducted.

When interpreting kidney outcomes in patients with FD, it is important to consider whether adjunctive therapies such as angiotensin converting enzyme (ACE) inhibitors, and angiotensin receptor blockers (ARBs) have been used because these can alter kidney outcomes.

4.3.1. Proteinuria and albuminuria

Proteinuria, specifically albuminuria, is the current gold-standard biomarker for Fabry nephropathy. In patients with classic FD, albuminuria usually emerges in the second or third decade of life [1,93]. ERT initiation is often delayed until significant albuminuria or glomerular filtration rate (GFR) decline occurs, a point where the reversibility of kidney damage is more difficult to achieve and prognosis is poorer [94], highlighting the importance of starting treatment early. Albuminuria, an important glomerular marker for proteinuria, is a sensitive biomarker to identify the early kidney complication of glomerular damage in FD, hence assessment of proteinuria levels without considering albuminuria levels could lead to a delayed diagnosis of Fabry nephropathy. Therefore, it is recommended to closely monitor albuminuria levels [95].

Evidence on whether there is a consistent effect of agalsidase alfa or agalsidase beta on albuminuria and proteinuria is mixed. Similarly, little evidence on the effect of migalastat on albuminuria was identified by this literature search, and caution is therefore warranted when interpreting the available data in regard to any modality of Fabry-specific treatment. One publication, reporting the effect of agalsidase alfa on proteinuria over 10 years, described stable levels in male patients (unreported phenotype) ≤30 years of age, but significant annual deterioration was observed in male patients above this age [96]. In this study, 38.4% of patients received at least one ACE inhibitor, so the stable proteinuria levels may be in part due to the use of this adjunctive therapy. Another publication reported the effect of different doses of ERT on albuminuria for up to 14 years in 20 patients with classic FD. The lower fixed dose group received agalsidase alfa or agalsidase beta 0.2 mg/kg EOW and the higher dose group received 0.2–1.0 mg/kg EOW. Podocyte GL3 reduction correlated with cumulative agalsidase dose ($r = 0.69$; $P = 0.001$), but no statistical difference in albuminuria from baseline was observed between the lower and higher dose groups. Of the 20 patients included in the study, 12 received a renin-angiotensin-aldosterone system blocker during some or all the follow-up period [97]. Two studies reported numerical increases of proteinuria levels; one study was in male and female patients (mixed phenotype) treated with either of the ERT preparations compared with migalastat [98], while the other study showed proteinuria levels were increased in male patients, correlated with reduced eGFR, but independent of receiving ERT [99,100]. One study reported a significant correlation between agalsidase beta and urinary excretion of urokinase-type plasminogen activator receptor in podocytes and urine protein/creatinine ratio (UPCR) [101]. One study on migalastat suggested greater increases in 24-h urinary protein levels in male patients with classic versus later-onset FD and compared with all female patients [100].

Evidence identified in this literature search suggests that ERT may have a beneficial effect on kidney function in terms of reduced albuminuria. However, as mentioned above, it is important to consider the effect of adjunctive therapies on kidney outcomes. A phase 3b trial identified a non-significant reduction in mean urine albumin:creatinine ratio (ACR) (mean change from baseline: −1.0 mg/g; $P = 0.0761$) in pediatric male patients with classic FD who were treated with agalsidase beta without the use of ACE inhibitors and ARBs, where no significant glomerulosclerosis was observed in kidney biopsies after 5 years [23]. Another study reported a direct correlation between urinary miR-21, belonging to the family of microRNAs, and degree of albuminuria in patients treated with ERT, suggesting both biomarkers are associated with kidney fibrosis and should be analyzed in a greater number of patients showing signs of pathological albuminuria. Although this publication did not report patient sex or FD phenotype, it did mention that the appearance of urinary microRNAs is a typical clinical manifestation of patients with classic FD [102]. Evidence identified in this study did not suggest that migalastat affected albuminuria levels in male or female patients with FD, although a phenotype-specific effect cannot be excluded because FD phenotypes were not reported [103].

Although proteinuria is frequently used as a kidney biomarker of FD, it has a relatively low sensitivity for the identification of early-stage nephropathy compared with low range albuminuria (ACR in microalbuminuria levels) and is limited as a predictor of kidney disease in female patients [21,104–106]. Therefore, the identification and validation of other markers of kidney impairment are important. One study compared albuminuria with other urinary biomarkers of glomerular and tubular dysfunction for the identification of early FD nephropathy. There was a significant increase in all biomarkers, even in the subgroup of patients with no evidence of nephropathy, overcoming the limitations of albuminuria as a sensitive marker of early kidney dysfunction [94,107].

4.3.2. Glomerular filtration rate

GFR, a marker of loss of kidney function due to advanced kidney injury [25], is one of the most important determinants of clinical outcome in patients with FD. The natural history of Fabry nephropathy in untreated patients varies substantially, with male sex, increased baseline proteinuria and reduced GFR all associated with more rapid decline in GFR. In patients with later-onset FD, or female patients with FD, GFR decline is generally slower and less predictable [108,109].

The gold-standard evaluation for GFR assessment is measurement by injection of an external marker (e.g., inulin, iohexol, chromEDTA, or iothalamate) followed by multiple blood samples, a procedure that is time consuming. Estimation of GFR by eGFR formulas based on serum creatinine and/or cystatin-C provides a more practical assessment of GFR in clinical practice. Creatinine is the parameter most used to evaluate for GFR estimation, but has limitations, including dependence on patient muscle mass. Cystatin-C is less subject to bias but is not available in all centers in part due to its higher cost [110–112]. Serum levels of

cystatin-C have been shown to be more sensitive than serum creatinine in detecting early kidney dysfunction and small reductions in GFR in both male and female patients [112], especially important in patients where muscle mass may vary due to different reasons such as pain in FD.

Consistent with previously published evidence, findings identified by this literature review show that ERT reduces the rate of decline in eGFR but the extent of the reduction can vary [19,25,39,51,96,98,100,113–116]. Most studies reporting an effect of ERT on eGFR were conducted in patients with classic FD, most of whom were male. Two studies found that eGFR decline was exacerbated in patients who had been switched from agalsidase beta to agalsidase alfa [25,51]. However, eGFR decline was attenuated in patients who had been switched from agalsidase alfa to agalsidase beta compared with those who had been switched from agalsidase alfa to agalsidase beta and then re-switched to agalsidase alfa [51]. This finding suggests that the extent of attenuated eGFR decline may be dose- and regimen-dependent. In another two studies [55,96], eGFR was quantified by using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation on the basis of serum creatinine (eGFRcreat), cystatin-C levels (eGFRcys), and a combination of creatinine and cystatin-C (eGFRcreat–cys). Findings from one of these studies suggested that agalsidase alfa slowed the rate of eGFR in male patients <30 years of age but not above this age [96]. The other study demonstrated a loss of eGFR in ERT-naïve females with FD after 12 months (112.3 vs 94.6 mL/min/1.73 m^2 for eGFRcys values at baseline and follow-up, respectively, and 92.2 vs 84.1 mL/min/1.73 m^2 for eGFRcreat values at baseline and follow-up, respectively) but not in ERT-naïve males. Out of 62 patients, only 5 (8%) patients presented with a mild ($n = 2$ [1 female]), or later-onset ($n = 3$ [1 female]) phenotype. The baseline characteristics were not different between ERT-naïve patients and those treated with ERT [55]. In the Lenders *et al.*, study, it is likely that patients with an unstable FASTEX score had a significant loss of kidney function [55].

Evidence identified by this literature review indicates comparable effectiveness of migalastat to ERT, in the patients with amenable mutations [98,116]. In one study of 14 patients, increase in enzymatic activity associated with reduced lyso-GL3 levels correlated with improvements in myocardial mass but not renal function [39]. Results from a recent *post hoc* analysis of 97 treatment-naïve and ERT-experienced patients with migalastat amenable *GLA* variants found no significant correlation between lyso-GL3 and eGFR in the overall patient group or subgroups stratified by prior ERT treatment status and sex [117]. As mentioned earlier, an important consideration when interpreting kidney outcomes in patients with FD is the use of adjunctive therapies such as ACE inhibitors and ARBs. These drugs can alter kidney outcomes and interpretation of eGFR because there may be a functional decrease of eGFR (as a result of reduced intraglomerular pressure due to vasodilation of the vas efferens), rather than structural damage to the kidney, and therefore therapeutic goals should be individualized.

4.3.3. Podocyturia

Kidney biopsies of children and adolescents with FD who had not been treated with ERT showed severe accumulation of granular lesions in podocytes, confirming that depositions of GL3 start in early childhood, long before overt clinical kidney disease [118]. Podocytes, which are a major target cell in Fabry nephropathy, accumulate α-Gal substrates, with morphological and functional alterations that may occur before the clinical evidence of proteinuria [104,105,119,120]. One study reported a significant positive correlation between agalsidase beta treatment and urinary excretion of urokinase-type plasminogen activator receptor in podocytes and UPCR in male and female patients, although FD phenotype was not specified [101].

Urinary loss of podocytes or podocyturia correlates with glomerular injury. It has been shown that podocyturia is inversely related to GFR in male patients and correlated with clinical severity in FD nephropathy, suggesting it could be a diagnostic tool and supporting its potential prognostic value in male patients with classic FD [104,105]. Despite podocyturia showing promise as an early indicator of kidney injury and use in diagnostic testing to assess kidney involvement, it is not generally assessed in routine clinical practice because the methods used to assess it are not yet standardized or widely available [110]. Longitudinal studies to further explore the effect of ERT or chaperone therapy on podocyturia, and other novel biomarkers of Fabry nephropathy, are warranted.

4.3.4. Summary of key recommendations for kidney biomarkers

Proteinuria, and specifically albuminuria, are biomarkers to support the diagnosis and assessment of patients with FD nephropathy [1,93]. Albuminuria is an important glomerular marker and a sensitive biomarker to identify the early kidney complication of glomerular damage in FD. GFR, a marker of advanced kidney injury, is useful in assessing treatment response and clinical outcomes in patients with FD [25]. ERT reduces the rate of decline in GFR, but the extent of the reduction varies among studies. Use of cystatin-C might improve measurement of kidney outcomes in patients with FD by providing a muscle-mass-independent criterion for early-stage FD nephropathy and guiding timely initiation of disease-specific treatment. Podocyturia could potentially support the diagnosis of FD and guide treatment strategies [104], and kidney biopsies may be helpful in clarifying the extent of GL3 accumulation and tissue damage, as well as supporting differential diagnoses [118].

4.4. Markers of immunogenicity and seroconversion

Therapeutic proteins have the potential to induce an immunogenic response and generate anti-drug antibodies (ADAs), especially when administered as multiple doses over prolonged periods [121,122]. ERTs contain α-Gal A proteins that are administered via intravenous infusion. More often in male patients than in female patients, therapeutic proteins may be recognized as foreign to the body and consequently can cause infusion-associated reactions (IARs) and the formation of ADAs [123]. IARs such as fever, chills, and chest pain, as well as the more classic immunological reactions such as edema, dyspnea, rash, itching, and, rarely, anaphylactic shock [124–127], can occur in patients with antibodies to ERT. There are few reports of immunoglobulin E (IgE) in patients treated with ERT, suggesting that IARs are IgE-independent and so mostly result from anaphylactoid reactions as opposed to anaphylactic reactions. Patients with ADAs against agalsidase are at a higher risk of IARs. Antibody status should be regularly monitored, particularly in males with a classic phenotype because the development of ADAs occurs almost exclusively in these patients [113]. ADAs can be neutralizing or non-neutralizing immunoglobulin G (IgG) antibodies and may impact on the overall clinical efficacy/effectiveness of ERT preparations, as they can inhibit α-Gal A activity, inhibit enzyme uptake into cells, have a negative impact on the pharmacokinetics of the exogenously administered enzyme, and limit the biochemical response to ERT [123,128–130].

The majority of studies identified in this literature search reported that ADAs to ERT were detected in male patients with a classic FD phenotype [9,88,103,115,131–133]. Overall, reports of increased ADA titers and consequent reductions in enzyme activity or plasma lyso-GL3 clearance have been described with both agalsidase alfa and agalsidase beta [9,12,88,103,115,129,131–133]. A negative correlation between increasing ADA titer against ERT and plasma lyso-GL3 clearance was demonstrated [113]. ADA development may also be ERT dose- and/or preparation-dependent [114], as one study identified an increase in seroconversion time associated with 1.0 mg/kg EOW versus 0.2 mg/kg EOW (unlicensed dose) of agalsidase beta [23], and another found an increased risk of ADA development associated with agalsidase beta compared with agalsidase alfa [12]. One study of 26 males with classic FD demonstrated a reduction in agalsidase alfa and agalsidase beta ADA

titer with high doses of immunosuppressive therapy due to kidney ($n = 24$) or heart ($n = 2$) transplantation [134]. Evidence also indicated statistically higher plasma lyso-GL3 in ADA-positive patients compared with ADA-negative patients [131], as well as attenuation of plasma lyso-GL3 response in ADA-positive patients [9,12,123].

In agreement with earlier publications [13], evidence identified by this literature review suggests an association between impaired plasma lyso-GL3 clearance and ADA-positive status [12,26,113,131,132]. Although lyso-GL3 reduction does not guarantee a clinical response in all affected organs, absence of a lyso-GL3 response after treatment initiation may suggest a loss of therapeutic effectiveness.

In the presence of ADAs against agalsidase alfa or agalsidase beta in male patients with classic FD, therapeutic non-saturation of ADAs was associated with a marked decrease in eGFR compared with those with saturation of ADAs [113]. In another study of 26 males with classic FD receiving ERT for a median of 94 months, a non-saturated ADA status during infusion was associated with progressive decrease in eGFR and ongoing cardiac hypertrophy. Dose escalation resulted in ADA saturation and reduced lyso-GL3 levels [114].

Increasing the dose of agalsidase alfa or agalsidase beta in male patients with classic FD with established inhibitory ADAs to saturate the ADAs has been shown to significantly reduce plasma lyso-GL3 levels [114]. Consistent with studies that pre-date the period covered, evidence identified by this literature review also suggests that switching from agalsidase alfa to agalsidase beta in ADA-positive patients may increase ADA titers but may also be associated with a reduction in plasma lyso-GL3 [25,135,136].

Some studies have also highlighted the possible benefit of immunomodulatory therapy in response to ADA formation, as immunosuppression has been shown to reduce ADAs significantly in patients who are receiving or have received ERT [103,121,134]. Routine antibody screening may inform treatment switching decisions. For example, if antibodies develop to either agalsidase alfa or agalsidase beta, a switch to migalastat could be an option in patients with amenable mutations, although this is currently not part of routine clinical practice. Additional evidence and consensus are needed to develop strategies relating to treatment switch, dose modification, and the potential use of immunomodulatory therapy.

4.4.1. Summary of key recommendations for markers of immunogenicity and seroconversion

Patients with FD, mostly males with a classic phenotype, receiving ERT may experience the formation of ADAs [113]. The findings identified in the literature review suggest that the presence of ADAs is associated with higher levels of lyso-GL3 and a decline in eGFR. Therefore, the potential loss of therapeutic effectiveness that is observed warrants routine measurement of ADAs in patients with FD, to guide treatment adjustment.

4.5. Central nervous system imaging markers

Clinical manifestations of FD often involve the CNS and can result in cognitive and psychiatric manifestations [137]. Cerebrovascular disease is prevalent in patients with FD and has a severe impact on patient QoL because it is highly debilitating, due to acute events such as stroke, and chronic impairment such as small vessel disease (SVD).

Common brain magnetic resonance imaging (MRI) findings associated with FD include white matter hyperintensities (WMH) and cerebral microbleeds (CMBs), which suggest the presence of cerebral SVD, and the pulvinar sign [138,139]. Individually, these neuroradiological markers lack sensitivity (the pulvinar sign) or specificity (WMH and CMBs). MRI-visible perivascular spaces (PVS) may be a marker of SVD that might be relevant for the diagnosis and understanding of the mechanism of white matter injury in FD. The study by Lyndon *et al.* found that when comparing MRI findings from patients with confirmed FD against healthy controls, and after adjustment for age and sex, FD was associated with more severe basal ganglia PVS, significantly higher WMH volume, more CMBs, and a higher prevalence of lacunes [140]. These findings were consistent with other studies confirming the association of SVD markers with FD [141,142], and in keeping with the functional relevance of PVS, given that cognitive impairment is common in FD [143,144]. In addition, another lysosomal storage disease, mucopolysaccharidosis, has also been associated with PVS enlargement [145]. Given that enlarged PVS occurs early in the course of SVD, before the burden of WMH increases, PVS may be an early indicator of FD cerebrovasculopathy [140,146].

Dolichoectasia of the basilar artery is an early finding in patients with FD and thus identification of dolichoectasic basilar arteries can be an early neuroradiological marker in patients with FD [147]. A study of the effect of ERT on basilar artery diameter in 30 male patients with FD with a mean duration of 7.2 ± 4.6 years until follow-up, using MRI, found a significant correlation between the duration of ERT and basilar artery diameter. This finding suggests that basilar artery diameter could be a potential surrogate marker of therapeutic efficacy of ERT. This was the only study identified in this literature review demonstrating the effect of ERT on basilar artery diameter; therefore, further studies are required to confirm these findings [148].

4.5.1. Summary of key recommendations for central nervous system biomarkers

Brain MRI can be performed in patients with suspected FD to potentially assist in the diagnosis of patients because findings such as WMH and CMBs may indicate signs of FD [138]. As the prevalence of the pulvinar sign is around 24% and is higher among males than females, its diagnostic value is limited [139]. Dolichoectasia of the basilar artery can also indicate FD, and therefore its identification can be an early neuroradiological marker in patients with FD [147]. Basilar artery diameter could also be a useful surrogate marker of therapeutic efficacy/effectiveness of ERT, as a finding from one study observed a correlation between duration of ERT and basilar artery diameter [148]; however, further investigation of this CNS marker is required to draw conclusive results.

5. Strengths and limitations

The publications identified in this literature review largely reflect outcomes in male patients with classic FD, so the biomarkers in these studies are primarily for that patient population. This especially highlights the need for further research into outcomes in female patients and those with later-onset disease. Many publications described outcomes for a mix of patients including male and female patients with classic disease, later-onset disease, or VUS, or did not specify whether patients had classic or later-onset disease. Therefore, the inclusion of patients with later-onset phenotypes in clinical studies looking at outcomes related to organs that are not affected in such patients could be a source of error. Furthermore, the analysis did not stratify the kidney or cardiac outcomes according to concomitant medications, which may be relevant considering the effect of medications such as ACE inhibitors and/or ARBs used to treat kidney and cardiac disease on the progression of FD nephropathy and FD cardiomyopathy. Data on CNS biomarkers are still limited, with the most important findings based on MRI.

6. Potential future biomarkers in Fabry disease

The challenge for the majority of potential biomarkers and why they are not all translatable to routine clinical practice is that the biomarker must have demonstrated technical, preclinical, and clinical validity, clinical utility must be clearly demonstrated, and biomarker measurement must have an impact on clinical management [149]. In addition, the search for new biomarkers and deeper understanding of FD may also

be limited by hypotheses-driven exploration of biological pathways that are already known to be associated with the disease. New biological signatures may enhance screening and diagnostic capability. In the post-genomic era, advances in omics technologies have generated abundant information that has advanced our understanding of complex inherited metabolic diseases. An omics approach also has the potential to overcome the limitations and potential biases of a hypothesis-driven biomarker search strategy [150]. A recent proteomics study that aimed to identify proteomic-based patterns that could distinguish between patients with FD, Pompe, Niemann-Pick type C, and Gaucher diseases, and healthy controls identified four discriminant proteins: fibroblast growth factor 2 (FGF2), VEGFA, VEGFC, and interleukin 7 (IL-7). However, no correlation was observed between these four proteins and lyso-GL3. In addition, a significant correlation between IL-7 and residual enzyme activity in a later-onset phenotype was identified, highlighting the multi-dimensional information an omics-based approach offers that may help in stratifying patients with FD with greater accuracy for improved clinical management [150]. Another avenue of exploration is the use of imaging data. This is a new biomarkers frontier and quantitative data extraction from imaging analysis remains a major challenge [17]. Development of novel imaging techniques, such as artificial intelligence (AI)-ECG, may provide solutions. Advancements in machine learning and computation methods have led to the clinical application of AI, which has the potential to improve diagnostic accuracy and efficiency by providing fully automated, unbiased, and unambiguous ECG analysis [151].

7. Conclusions

The gold standard of FD diagnosis is the assessment of α-Gal A activity (for males) and confirmatory genetic analysis but identification of the broad range of variants is challenging. Disease-specific biomarkers such as lyso-GL3 may be valuable additions to current FD diagnostic procedures and aid in objective monitoring of pharmacodynamic response in patients with classic FD. Lyso-GL3 has been shown to decline in response to ERT but there is not a clear correlation between the change in lyso-GL3 levels with a reduction in clinical events. In addition, lyso-GL3 only partially correlates with response to chaperone therapy. Furthermore, the value of lyso-GL3 as a biomarker in patients with a later-onset FD phenotype is still not well understood, nor do we have sufficient data on changes in lyso-GL3 levels as patients age. Non-disease-specific biomarkers are of value in assessing organ involvement but should be used in conjunction with disease-specific biomarkers to support their use in monitoring response to treatment. In addition, assessment of ADAs and imaging studies, including CMR T1 mapping, may be valuable tools to monitor therapeutic response and disease progression in patients with FD, but their widespread application is hampered by limited availability across all countries. The future of biomarker discovery in FD may lie in the exploration of biological pathways that have not been considered, such as the FGF2 signaling and inflammatory cytokine signaling pathways [150]. In conjunction with biomarkers that are already being studied in FD, the identification of novel molecular signatures may enhance our understanding of the complex underlying pathophysiology, thereby providing opportunities for therapeutic innovation.

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Data availability

Data will be made available on request.

Declaration of Competing Interest

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Appendix A. Supplementary data

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