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Abstract

Marfan Syndrome is an autosomal dominant, genetically inherited connective tissue disorder which primarily affects the cardiovascular system but can also have systemic manifestations. First described in 1896, Marfan Syndrome has a prevalence of around 1/5000 in the general population. It is becoming increasingly common to see patients with Marfan Syndrome in a clinical setting due to the improved care of patients with adult congenital heart disease and general improvement in survival. Mortality however remains high, largely due to the risk of aortic dissection as a result of the aortic root dilatation frequently seen in these patients. Contemporary management therefore has been focused on imaging-based surveillance to prevent these catastrophic events and intervene surgically in a timely manner. However, it is increasingly recognised that some patients do suffer aortic dissection below the expected threshold for surgical intervention. With this in mind, there has been interest in the role of biomarkers as an adjunct to imaging in the care of these patients. This article will provide an overview of the literature on potential biomarkers studied so far in Marfan Syndrome as well as potential future directions.

Introduction

Marfan Syndrome – a multi-system disorder with phenotypic variability

Marfan Syndrome (MFS) is an autosomal dominant inherited connective tissue disease with an estimated prevalence of 1/5,000 in the general population.[1] It is a disease with multi-system involvement and phenotypic variability. First described by Antoine-Bernard Marfan in 1896, it is caused by a mutation in the FBN1 gene on chromosome *15q21* encoding fibrillin-1.[2,3] Fibrillin-1 is a large extracellular matrix glycoprotein that provides elasticity as well as structural integrity to connective tissues. Although the typical clinical features involve cardiac, skeletal, and ocular systems, the main risk of morbidity and mortality in these patients is attributed to the development of aortopathy and subsequent aortic dissection. Aneurysm of the ascending aorta was first described by Taussig and colleagues in 1943.[4] Aortic dissection often presents acutely with a high risk of mortality and is not always preceded by chronic symptoms. For this reason, the current European Society of Cardiology (ESC) guidelines for the management of grown-up congenital heart disease recommends yearly echo surveillance.[5] Aortic root surgery is recommended once the maximal aortic root diameter reaches ≥ 45 mm in those who are at high risk.[5] The average life expectancy for patients with MFS is estimated to be around 41 years.[6]

Fibrillin-1 and the role of TGF-ß

The connective tissue disorder seen in MFS is caused by mutations in fibrillin-1 which is encoded by the FBN1 gene.[7] Fibrillin-1 is a 350kD glycoprotein that is synthesized as a 375 kD precursor and is processed and secreted into the extracellular matrix (ECM).[8] Fibrillin monomers polymerise into microfibrils that

incorporate other proteins, for example latent transforming growth factor ß-binding proteins (LTBPs).[8] This fibrillin-LTBPs complex binds TGF-ß in an inactive state. Mutations in fibrillin-1 therefore disrupts the relationship between fibrillin-1 and LTBPs. The result is diminished sequestration of latent TGF-ß in the ECM and subsequent excess TGF-ß signalling.[7] It has been shown that excess signalling and altered TGF-ß activation lead to the pathological changes in the aorta and aortic root dilatation seen in MFS. This concept has been demonstrated in animal models and more importantly, the administration of TGF-ß neutralising antibodies prevented these changes.[9] It has formed the basis of our understanding of MFS and led to the development of clinical trials utilising angiotensin-receptor blockers and beta-blockers in treating patients with MFS.[10-15]

Clinical utilities of biomarkers in MFS – the rationale for a biomarker

Despite echo-based surveillance being the current mainstay of management, challenges remain. Firstly, echo surveillance is operator and patient dependent.[16] A larger body habitus attenuates ultrasound waveforms and often reduces the quality of images obtained, resulting in reduced accuracy of any subsequent measurements obtained. A more experienced operator is also more likely to obtain better images for analysis. Although cross-sectional imaging such as computed tomography (CT) offers higher resolution images and therefore more accurate measurements, it utilises more radiation and therefore is unsuitable for regular use in MFS patients who are predominantly young individuals requiring prolonged periods of surveillance. Secondly, aortic dissection can still occur in between scheduled surveillance scans due to the highly variable onset and rate of aortic dilatation.[17] For example, a period of quiescent growth for many years can be followed by a period of rapid growth

within a short space of time. Thirdly, it is noteworthy that aortic dissection can still occur below the surgical threshold. In a published series of 524 MFS patients, 158 patients had aortic dissection and 46% of these were below the surgical threshold.[4] This suggests imaging-based surveillance alone in a disease which has a variable rate of progression is an unreliable predictor of risk of dissection and may not accurately reflect the degree of disease severity.[18]

Due to this, there has been recent interest in the role of biomarkers as potential adjuncts to imaging in order to improve risk stratification and predict the course of the disease. For example, augmentation index – a marker of arterial stiffness measured non-invasively by applanation tonometry – has been shown to independently predict progression of aortic root disease.[19-21] Similarly, the role of circulating biomarkers to predict rate of aortic dilatation and improve risk stratification has been explored. In a disease such as MFS where there is a highly variable age of onset, phenotype, and rate of aortic dilatation, a biomarker with good predictive value could potentially be useful. Moreover, not all FBN1 gene mutations lead to a diagnosis of MFS.[2] Although the study of biomarkers in MFS is not an entirely evidence-free zone, to our knowledge there has been very limited previous work on circulating biomarkers in this patient group apart from studies on TGF-β. Crucially, no biomarker has made the successful transition into routine clinical use. This is likely due to the inherent challenges of an ideal biomarker, which ideally should be pathologically relevant, measurable, has prognostic value or able to measure response to therapy. In this review, we summarise previously studied plasma biomarkers (Table 1) in particular TGF-β as well as other potential biomarkers that have been studied in other forms of aortopathies (Table 2) and therefore might potentially prove useful in MFS.

Biomarker candidates in MFS – circulating biomarkers

1. **TGF-β**

Due to the central role that excess TGF-β signalling plays in MFS, it is unsurprising that there has been great interest in its potential role as a plasma biomarker and it remains the most studied out of all potential biomarkers. In a study of 99 MFS patients, Lutter at al demonstrated significantly higher circulating plasma levels of TGF-β in MFS patients compared to healthy controls (109 pg/ml vs 54 pg/ml, p<0.001).[22] A higher plasma TGF-β level was also modestly correlated with larger aortic root dimensions measured on magnetic resonance imaging (MRI) (r = 0.26, p=0.027), previous aortic root surgery and faster aortic root growth rate (r=0.42, p<0.001).[22] The optimal cut-off value was 140 pg/ml (AUC 0.71, 95% CI 0.58 to 0.84, p = 0.006) for the composite clinical endpoint of aortic dissection and prophylactic aortic root replacement. Patients with plasma TGF-β levels beyond this threshold were 6.5 times more likely to experience the composite endpoint.[22]

This finding echoes earlier findings in murine models which have been validated in human MFS subjects. Circulating plasma TGF-β1 levels were observed to be higher in 16 MFS mutant mice (*Fbn1$^{C1039G/+}$*) compared to wild-type mice (115 ± 8 ng/mL versus 92 ± 4 ng/mL, p=0.01).[23] Correlation was seen between circulating free TGF-β1 levels and aortic root diameters at the level of Sinus of Valsalva (r=0.6, p<0.001). In human subjects, TGF-β1 was also seen to be significantly elevated in patients with MFS (n=53) compared to controls (15 ± 1.7 ng/mL versus 2.5 ± 0.4 ng/mL, p<0.0001).[23] It is noteworthy that circulating TGF-β1 levels were seen to decrease after the administration of losartan and ß-blocker therapy, reflecting the benefit of blunting excessive TGF-ß activation.[23]

Despite this, it is recognised that TGF-β response to angiotensin receptor blockers can be varied. In a sub-study of an open-label randomised controlled trial comparing losartan to standard therapy (COMPARE trial), the investigators reported only a third of patients had a reduction in TGF-β despite having a reduction in aortic dilatation rate (AoDR).[24,25] Patients who demonstrated a reduction in TGF-β levels following losartan therapy had higher baseline plasma TGF-β and a higher AoDR.[24] This suggests TGF-β levels are probably markers of aortic disease severity rather than initiators of aortic dilatation.[24] Variability of TGF-β levels following therapy raises uncertainty with regards to its value in monitoring treatment response.

In addition, not all studies show increased levels of TGF-β in MFS patients. Ogawa and colleagues did not find a significant difference in TGF-β1 levels in a cohort of Japanese MFS patients compared to controls.[26] This finding is different to that of earlier studies of TGF-β. Although this alone does not eliminate the possibility of its role in MFS pathogenesis, it is possible that there are ethnic and genetic differences affecting levels of TGF-β in MFS as the majority of earlier studies were conducted in white European/north American cohorts.

Therefore TGF-β remains a potential biomarker and has not yet made the successful transition into routine clinical practice. A few possible explanations exist. Firstly, there is a huge number of possible FBN1 mutations. The most recent update lists 3077 possible mutations.[27] Depending on the underlying mutation, either a dominant negative or haploinsufficient effect will be seen and will affect the amount of TGF-β released from the fibrillin-1 network.[24] Secondly, there is a variation in the abundance

of angiotensin type 1-receptor (AT-1) expression. Finally polymorphism in the renin-angiotensin-aldosterone system (RAAS) could also affect TGF-β response.[24]

2. Fibrillin-1 fragments

FBN-1 mutations lead to instability in the fibrillin-1 protein and therefore increases its susceptibility to proteolytic degradation.[28] Marshall and colleagues hypothesized that proteolytic degradation releases fragments of fibrillin-1 and other elastic fibre proteins into the circulation and can therefore be used as a potential biomarker, specifically as a predictive tool for risk of aortic dissection in MFS and other thoracic aortic aneurysms (TAA).[28] In a study of 1265 patients from a registry cohort, 118 of whom had a confirmed or suspected diagnosis of MFS, plasma levels of fibrillin-1, fibrillin-2 and fibulin-4 were measured and compared to controls. A significantly higher proportion of patients with TAA had detectable fibrillin-1 fragments. Acute or subacute aortic dissection were associated with fibrillin-1 levels in the highest quartile, compared to patients with no dissection or those with chronic dissection.[28] These associations were not seen with fibrillin-2 or fibulin-4.

In addition, fibrillin-1 levels were significantly associated with aortic aneurysm location. Patients with TAA demonstrated higher levels of measurable fibrillin-1 segments compared to those with abdominal aortic aneurysm (AAA).[28] Based on this early work, further longitudinal studies to assess the relationship of circulating fibrillin-1 levels and long-term rate of aortic dilatation rate are ongoing.

3. Homocysteine Y (tHCy)

The large variation in cardiovascular manifestations among MFS patients is well recognised. Giusti et al sought to explain this observation by exploring the role

played by homocysteinaemia and the prevalence of the C677T methylenetetrahydrofolatereductase (MTHFR) polymorphism.[29] Plasma homocysteine has previously been shown to correlate significantly with the degree of atherosclerosis in the thoracic aorta.[30] Furthermore, higher levels of plasma homocysteine Y (tHCy) were also shown to be associated with a higher risk of requiring AAA surgery, suggesting a potential role played by tHCy in endothelial disruption.[2]

Giusti and colleagues therefore explored whether this same relationship existed in MFS patients. 107 MFS patients were divided into 3 groups based on whether they had no cardiovascular manifestations, mild cardiovascular manifestations (defined as aortic root dilatation < 2.2 cm/m^2 body surface area) or major cardiovascular manifestations (aortic root dilatation > 2.2 cm/m^2 body surface area). Following multivariate logistic regression analysis, the authors concluded that there was a significantly higher level of plasma tHCy in MFS patients with more severe cardiovascular manifestations compared to those who do not. Interestingly, levels of tHCY also reflected disease acuity as within this same group those with aortic dissection had the highest level of tHCy. This could potentially be explained by increased elastolysis via MMP activation that is induced by hyperhomocysteinaemia as shown in previous studies.[31,32] Large, prospective studies with hard clinical outcomes or long-term follow-up are lacking and therefore its real world clinical utility remains unclear.

4. Matrix metalloproteinase (MMP)

Matrix metalloproteinases are endopeptidases which play a key role in the elastolysis of the ECM in MFS. It has been established in previous studies that the

process of medial degeneration in thoracic aortic aneurysm of MFS patients is facilitated by the increased levels of MMP activity.[33] In addition to cystic medial necrosis (CMN), immunohistochemistry of tissue samples from MFS patients with aortic aneurysm have shown strong immunoreactivity for all MMP subtypes, in particular MMP-2 and MMP-9.[33,34] Upregulation of MMP-2 and MMP-9 activity result in loss of elastic fibre integrity and reduction of vascular smooth muscle (VSMC) contraction.[34,35] In mice models of MFS, deletion of MMP-2 inhibited TGF-β activation and phosphorylation of Erk1/2 and Smad2 resulting in prolonged lifespan of the mice.[34]

Although the role of MMP as a plasma biomarker in MFS has not been studied, it has been studied in patients with bicuspid aortic valve (BAV) aortopathy. BAV has similarities with MFS with regards to deficiency in fibrillin-1 content within the aortic wall as demonstrated by Fedak and colleagues in a study of 16 BAV patients where fibrillin-1 content within the aortic tissue of BAV patients were found to be significantly less than in patients with tricuspid aortic valves.[36]

Studies of MMP in BAV patients have demonstrated increased levels of circulating MMP particularly MMP-2 and MMP-9, however the relationship with tissue expression of MMP has been less certain. In a study of 29 patients with ascending aortic aneurysms, 14 of whom had BAV, LeMaire et al. demonstrated normal tissue MMP-9 expression in patients with BAV-associated aortopathy, a finding that was different to Boyum et al. who showed significantly increased tissue MMP-9 concentrations in patients with BAV aortopathy.[37,38] This reflects the heterogeneity in phenotype seen in BAV, a feature that is also more prevalent in MFS. The majority of

studies of MMP levels in BAV patients have focused on its correlation with aortic root size and although this has been shown, there has been no study so far that has demonstrated its prognostic predictive value for adverse events.[39]

What is clear however, higher levels of MMP-2 in patients with BAV and a dilated proximal aorta has been associated with endothelial dysfunction and increased aortic stiffness compared to those without proximal aortic dilatation.[40] These are positive early findings in the BAV cohort, and they have not been extensively studied in MFS patients.

5. Lysyl oxidase

Elastin provides structural integrity to the ECM and elasticity for arterial structures such as the aorta. Mature elastin is formed from precursor tropoelastin molecules which are linked via their lysine residues with desmosine and isodesmosine cross-links. This process is catalysed by lysyl oxidase which is an extracellular, copper-containing enzyme.[41] It is encoded by the LOX gene and inactivation has been shown to cause structural alteration in abdominal arterial walls.[41,42] Therefore, high levels of LOX gene expression may stabilise an aneurysm, and LOX isoforms are measurable in serum samples.[2] However, these studies have been based on murine models of AAA as well as other diseases such as systemic sclerosis and certain types of tumours. Its role as a biomarker in human MFS has not yet been studied and is therefore unclear.[41,42]

6. Tissue inhibitor of MMP (TIMP)

Previous immunohistochemistry studies of tissue samples from patients with MFS have demonstrated cystic medial necrosis and loss of elastic fibres but more importantly also demonstrated increased levels of expression of MMPs without corresponding increase in TIMPs. This imbalance of activity between MMPs and their endogenous inhibitors - tissue inhibitor of MMP (TIMP) - has been implicated in the development of aortic root dilatation seen in MFS.[43,44] Studies exploring its potential use as a biomarker however are limited.

7. Collagen and elastin markers

Markers of elastin and collagen degradation have been studied in AAA patients but not in patients with MFS. However, it is possible that similar findings will be present in MFS and patients with TAA due to the role of elastin degradation in aneurysm dilatation and collagen breakdown leading to rupture. In a study of 87 patients with AAA, serum propeptide of type III procollagen (PIIINP), a marker of type III collagen turnover was found to be higher than in control subjects (3.47 μg/L vs 2.73 μg/L, p<0.0001).[45] There was only a weak correlation with AAA diameter (r = 0.27, p=0.04).[45]

Elastin turnover and collagen degradation might also affect aortic distensibility, a factor that may predict aortic aneurysm growth and rupture. This hypothesis was explored by Wilson et al. by measuring serum elastin peptides (SEP), plasma elastin-α_1-antitrypsin complex (E-AT), and PIIINP; all markers of serum elastin and collagen metabolism. These were compared to aortic distensibility measured on ultrasound by means of pressure-strain elastin modulus (Ep) and stiffness.[46] Aortic wall distensibility was associated with increased elastin turnover but was inversely related with collagen turnover.[46] This suggests that an aortic aneurysm becomes less

compliant and less able to cope with haemodynamic stress as it grows and loses increasing amounts of collagen.

8. Genetic biomarkers

The potential use of genetic sequencing to identify common loci associated with familial TAA in MFS and use it as a biomarker is enticing. Recent work have identified the potential of utilising non coding micro-RNA as a potential therapeutic or biomarker target.[47] The technology however is still developing and it remains to be seen if this will be translated into clinical practice.[47] In addition, genetic biomarkers are unlikely to be able to monitor disease progression due to the fixed nature of the genomic code. It is also common to find a similar genetic variant among different types of hereditary TAA, rendering this method less specific for MFS. Costs are potentially a barrier to widespread clinical use.

Biomarker candidates in MFS – imaging biomarkers

Apart from the aforementioned potential circulating biomarkers, there has been previous work on imaging biomarkers, albeit limited. In this regard, magnetic resonance imaging (MRI) is the most studied modality as a potential adjunct to echocardiography.

The role of MRI as a potential prognostic imaging biomarker has been explored by Morris et al. who demonstrated that increased vertebral tortuosity index (VTI) as measured by magnetic resonance angiography (MRA) is a reproducible marker of adverse cardiovascular outcomes in patients with connective tissue disorders.[48] Their

analysis on patients with MFS and Loeys-Dietz Syndrome (LDS) revealed a higher risk of earlier surgery for those patients with a higher VTI compared to controls, after adjusting for aortic root size.[48] There was no significant correlation between VTI and change in aortic z-score over time.[48] Vertebral tortuosity was a common finding in MFS patients, reflecting multi-system involvement beyond aortic root and proximal ascending aorta. The authors hypothesised that vascular fragility as a result of defective fibrillin-1 manifests itself as tortuosity in the thin-walled vertebral arteries, although this has not yet been proven.[48]

Similarly, a higher aortic tortuosity index (ATI) obtained by MRI has been shown by Franken et al. to be associated with a more severe MFS aortic phenotype and an increased risk of developing an aortic dissection.[49] However, to the best of our knowledge none of these imaging techniques have made it into routine clinical use as a prognostic biomarker or to monitor therapy.

Future perspectives

The inability of previously studied biomarkers to make the transition to routine clinical use creates an unmet clinical need in this area. Phenotype heterogeneity in congenital heart disease such as MFS makes finding a pathologically relevant plasma biomarker challenging. MFS aortopathy is understood to involve many different complex mechanisms, as discussed previously and an ideal plasma biomarker should be measurable, reliable, and more importantly pathologically relevant. There is currently a lack of prospective studies or large MFS registries to test the clinical utility of potential biomarkers.

Recently, there has been interest in newer biomarker candidates. In particular, biomarkers that reflect the process of elastin degradation which is a common pathway shared by all forms of aortopathies. In this regard, the role of desmosine which is a specific elastin-degradation product has been explored as a potential biomarker in AAA and MFS. Early work on a cohort of MFS patients have yielded encouraging results.[50] Plasma and urinary desmosine were significantly elevated compared to age and gender-matched controls.[50] Urinary desmosine (uDES) also showed a strong correlation with aortic root size (r=0.79, p=0.04).[50] Further work is therefore needed in this area utilising larger cohorts of patients to determine its prognostic value.

Conclusion

With advances in care for patients with adult congenital heart disease (ACHD), the majority of patients are surviving well into adulthood. Mortality in patients with MFS remains high and aortic dissection resulting from aortopathies remain the biggest contributing factor. Biomarkers have a potential adjunctive role to play, in monitoring disease progression as well as identifying those at greatest risk of adverse events. TGF-β remains the most studied out of all the potential biomarkers, however none have made the successful transition into routine clinical practice. This is likely due to the inherent challenges of identifying an ideal biomarker, requiring it to be pathologically relevant, has prognostic value, or able to monitor response to treatment. Newer biomarker candidates therefore have a significant role to play and further work is needed in this area of unmet clinical need.

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Biomarker	Key findings	Proposed utilities	Original publication
TGF- ß	Plasma TGF- ß correlates with larger aortic root diameters, faster aortic root growth and earlier aortic root surgery for levels > 140 pg/mL	Predictor for aortic root size, rate of growth and cardiovascular events	Franken et al[22]
	Total serum TGF- ß1 is increased in patients with MFS with causative FBN1 mutations		Hillebrand et al[51], Matt et al[23]
	Losartan responders had higher baseline plasma TGF- levels		Franken et al[24]
	Plasma TGF- ß levels were significantly higher in MFS patients with aortic root dilatation compared to those without		Radonic et al[52]
	No significant difference in plasma TGF-β1 levels between MFS patients and controls in a Japanese population		Ogawa et al[26]
	TGF-β1 expression in peripheral blood and aneurysmal aortic tissues was significantly elevated in MFS patients		Kim et al[53]
Fibrillin-1	Circulating fibrillin-1 fragments were higher in patients with TAA	Marker of disease severity	Marshall et al[28]
Total homocysteine (tHcy)	tHcy levels were significantly higher in patients with more severe cardiovascular manifestations of MFS and aortic dissection compared to patients with milder phenotypes and no aortic dissection	Marker of disease severity	Giusti et al[54]
TIMP	Imbalance between MMP and TIMP activity in MFS subjects	Mechanistic explanation for ECM remodelling seen in MFS. Potential therapeutic target.	Williams et al[43] Ikonomidis[44]

ECM, extracellular matrix; FBN1, fibrillin-1; MFS, Marfan Syndrome; MMP, matrix metalloproteinase; TAA, thoracic aortic aneurysm; TGF- ß, transforming growth factor beta; tHCY; total homocysteine; TIMP, tissue inhibitor of metalloproteinase

Table 1 - Previously studied plasma biomarkers of aortopathy in MFS

Biomarker	Key findings	Proposed utilities	Original publication
MMP	Higher circulating levels of MMP, particularly MMP-2 and MMP-9 have been demonstrated in multiple BAV cohorts	Marker of aortic dilatation	Drapisz et al[55], Wang et al[39], LeMaire et al[38], Tzemos et al[40], Ikonomidis et al[56], Boyum et al[37]
Lysyl oxidase	Encoded by LOX gene, high level of gene expression stabilises AAA	Therapeutic use of overexpression of LOX/LOXL enzymes to stabilize AAA	Joni et al[41], Remus et al[42], Pepe et al[2]
Serum propeptide of type III procollagen (PIIINP)	Serum PIIINP higher in AAA patients than in controls	Marker of aortic dilatation	Satta et al[45]
Serum elastin peptides (SEP), plasma elastin-α1-antitrypsin complex (E-AT)	Both SEP and plasma E-AT correlated with higher elastin turnover and decreased aortic compliance	Predictor of rupture	Wilson et al.[46]

AAA, abdominal aortic aneurysm; BAV, bicuspid aortic valve; E-AT, plasma elastin-α1-antitrypsin complex; LOX, lysyl oxidase; LOXL, lysyl oxidase homolog; MMP, matrix metalloproteinase; PIIINP, serum propeptide of type III procollagen; SEP, serum elastin peptides;

Table 2 - Previously studied plasma biomarkers of aortopathies not related to MFS with potential use in MFS