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Original Article

Aortic Dimensions, Biophysical Properties, and Plasma Biomarkers in Children and Adults with Marfan or Loeys-Dietz Syndrome

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ABSTRACT

Background: Aortic dilation, stiffening, and dissection are common and potentially lethal complications of Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS), which involve abnormal transforming growth factor beta (TGF-β) signalling. The relation of aortic dimensions, stiffness, and biomarker levels is unknown. The objective of this study was to measure aortic dimensions, stiffness, TGF-β and matrix metalloproteinase (MMP) levels, and endothelial function in patients with MFS, and to compare TGF-β levels in patients with MFS receiving different therapeutic regimens.

RÉSUMÉ

Contexte : La dilatation, la rigidification et la dissection de l'aorte sont des complications fréquentes et parfois mortelles du syndrome de Marfan (SM) et du syndrome de Loeys-Dietz (SLD), qui sont tous deux dûs à une anomalie de la voie de signalisation du facteur de croissance transformant bêta (TGF-β). On ne connaît pas la relation entre les dimensions et la rigidité de l'aorte et la présence de biomarqueurs. Notre étude visait à mesurer les dimensions et la rigidité de l'aorte, les taux de TGF-β et de métalloprotéases matricielles (MMP) et la fonction endothéliale chez des patients atteints du SM, et à les comparer aux

Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS) are hereditary aortopathies resulting from abnormal transforming growth factor beta (TGF-β) signalling.[1,2] The clinical features of both are pleiotropic and they share phenotypic features and, most significantly, aortic dilation and dissection leading to early death.[3] However, with recent improvements in the management of MFS, the average life expectancy has increased to almost normal.[4] The abnormal cellular signalling mechanisms in these syndromes are complex.[5-7] The prevailing theory stipulates that fibrillin-1 mutations cause the release of fibrillin-1-bound latent TGF-β, resulting in increased levels of circulating TGF-β, which initiates intracellular cascades with downstream effects that account for microfibrillar proteolysis, elastin breakage leading to aortic dilatation, dissection, and other clinical changes observed in these conditions.[8,9] Increased

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Methods: This was a cohort study of 40 MFS and 4 LDS patients and 87 control participants. Aortic dimension and stiffness indexes, including pulse wave velocity (PWV), were measured using echocardiography and Doppler. Total and free TGF-β and MMP blood levels were measured using Quantikine (R&D Systems, Inc, Minneapolis, MN) and Quanterix (Billerica, MA) kits. Endothelial function was measured using brachial artery flow-mediated dilation.

Results: PWV was increased in patients with MFS. There were increased MMP-2 levels in those with MFS but no increase in free or total TGF-β or MMP-9 levels compared with control participants. There was no difference in TGF-β levels between MFS patients receiving no medications, angiotensin receptor blockers, and β-blockers. PWV correlated most strongly with age. Endothelial function showed premature gradual decline in patients with MFS.

Conclusions: Despite the increased PWV, monitoring aortic stiffness or TGF-β levels would not be helpful in patients with MFS. TGF-β levels were not increased and the increased MMP-2 levels suggest consideration of a different therapeutic target.

Méthodologie : Il s'agissait d'une étude de cohorte menée auprès de 40 patients atteints du SM et de quatre patients atteints du SLD, ainsi que de 87 témoins. Les indices des dimensions et de la rigidité aortiques, y compris la vitesse d'onde de pouls (VOP), ont été mesurés par échocardiographie et par échographie Doppler. Les taux sanguins de TGF-β et de MMP totaux et libres ont été mesurés à l'aide de trousses Quantikine (R&D Systems, Inc, Minneapolis, MN) et Quanterix (Billerica, MA). La fonction endothéliale a été mesurée par dilatation liée au flux dans l'artère brachiale.

Résultats : La VOP était plus élevée chez les patients atteints du SM. On a aussi observé une hausse des taux de MMP-2 chez les patients atteints de SM, mais aucune augmentation des taux de TGF-β ou de MMP-9 libres ou totaux comparativement aux témoins. Il n'y avait pas de différence entre les taux de TGF-β chez les patients atteints de SM ne recevant aucun traitement, ceux qui prenaient un antagoniste des récepteurs de l'angiotensine et ceux qui prenaient un bêtabloquant. La VOP été plus fortement corrélée avec l'âge. La fonction endothéliale a affiché un déclin progressif prématuré chez les patients atteints du SM.

Conclusions : Malgré l'augmentation de la VOP, il ne semble pas utile de surveiller la rigidité aortique ni les taux de TGF-β en cas de SM. Les taux de TGF-β n'étaient pas plus élevés chez les patients atteints du SM, et la hausse des taux de MMP-2 indique qu'il conviendrait de choisir une autre cible thérapeutique.

levels of circulating TGF-β have been found in patients with MFS and to a lesser extent in those receiving medical treatment.[10,11] It has been proposed that circulating TGF-β might serve as a prognostic biomarker for aortic disease and aid in the decision for elective surgery. However, no association between aortic root size and circulating TGF-β was reported in a Japanese study of MFS patients,[12] and aortic dissection has been reported in some MFS and LDS patients with a low-risk aortic root dimension.[13] The primary role of TGF-β signalling in the causation of aortic aneurysms in these genetic syndromes has been challenged recently.[14,15] Other common biomarkers have not been extensively studied in these conditions although abnormal matrix metalloproteinase (MMP) levels have been reported in a mouse model of MFS; changes in apolipoprotein levels have been detected in animal studies and in patients with abdominal aneurysms. However, the mechanistic significance of potential biomarkers has not been established.[6,16,17]

In addition to aortic dilatation, human and animal evidence shows increased aortic wall stiffness in those with MFS, and dysfunction of smooth muscle and endothelial cells.[7,18-20] Echocardiography is used for monitoring of aortic root size, and can be used to assess pulse wave velocity (PWV), stiffness, and vascular impedance.[21,22] Increased aortic stiffness has been shown in MFS patients even in the absence of aortic dilatation.[19] Unfortunately, to our knowledge, there have been no studies to investigate whether serum biomarker levels correlate with the biophysical properties of the aorta in patients with these forms of genetic aortopathy or whether monitoring these might be clinically useful. Hence, the purpose of this study was to measure the aortic root dimensions, the biophysical properties of the aorta, and serum biomarkers and determine if there is any correlation in patients with MFS and LDS.

Methods

Patients

The study protocol was approved by the University of British Columbia Clinical Research Ethics Board and the Children's and Women's Health Centre of British Columbia's Research Review Committee. Consecutive patients who met the criteria for MFS on the basis of the Ghent nosology[23] were recruited at British Columbia Children's Hospital and St Paul's Hospital, in Vancouver, Canada. All patients underwent physical examination and genetic screening before enrollment. The inclusion criteria were: subjects must (1) conform to the diagnostic criteria for MFS or LDS; (2) be between 8 and 60 years old; (3) had not undergone aortic root surgery; (4) have technically suitable echocardiographic windows to obtain the required images; and (5) provide informed consent and/or assent. The exclusion criteria were: subjects (1) with significant aortic or mitral valve regurgitation; (2) who were pregnant; and (3) who use illicit drugs. We did subgroup analyses on patients younger than 20 years and older than 20 years. Patients were matched 1:2 to healthy control participants from our institutional database and older subjects (older than 20 years) recruited prospectively who met the following criteria: subjects must (1) be between 8 and 60 years old; (2) be in good health with no acute or chronic illness; (3) have technically suitable echocardiographic windows to obtain the required images (imaging was attempted in all potential participants); and (4) provide informed consent. The control subject exclusion criteria were: subjects who (1) had undergone cardiothoracic surgery or whose aortic dimensions were abnormal; (2) were pregnant; and (3) use nicotine products or illicit drugs.

After consent was obtained, height, weight, arm span, blood pressure (BP), medication history, and genetic test data were recorded. All patients had full echocardiography. Flow-mediated dilation (FMD), was performed in all but 7 patients with MFS. Nitroglycerin challenge was performed in adults who met the BP criteria of systolic BP of 100 mm Hg or more. Blood for biomarkers was obtained in all but 5 MFS patients, and adult control subjects who consented to blood analyses. The interpreting physicians and laboratories were blinded to the patient's identity and disease or control category.

Echocardiography

Standard 2-dimensional, M-mode, and Doppler echocardiography was performed on all patients. Left ventricular volumes, ejection fraction, aortic dimensions, and cardiac output were calculated. Our laboratory protocol for measuring the biophysical properties of the aorta was undertaken as described previously.[19] The methods and calculations used to derive all of the indexes of vascular function are described in Supplemental Appendix S1.

Endothelial function

Brachial artery FMD was performed according to the standard protocol. The images were analyzed by the Cardiovascular Imaging Research Core Laboratory at the Vancouver Hospital and Health Sciences Centre.

Blood sample collection and processing

The blood sample collection, processing, and storage protocol has been previously described.[24] Freeze-thaw cycles were avoided to ensure the accuracy and reliability of the measurements.

Plasma biomarker measurement using enzyme-linked immunosorbent assay

For the quantitative determination of circulating levels of TGF-β1, standard enzyme-linked immunosorbent assay (ELISA) testing was performed using a commercially available kit, Human TGF-β1 Quantikine ELISA Kit DB100B (R&D Systems, Inc, Minneapolis, MN). MMP-2 and MMP-9 concentrations were measured following similar procedures for TGF-β1, except for the sample activation, using Total MMP-2 Quantikine ELISA Kit MMP200 (R&D Systems, Inc) and Human MMP-9 Quantikine ELISA Kit DMP900 (R&D Systems, Inc), respectively. The plasma levels of TGF-β1 and MMP-2/-9 of 44 patients were compared with that of 37 healthy control participants.

To comply with the suggestion that free TGF-β1 should be measured,[25] free and total TGF-β1 were measured using a Simoa HD-1 analyzer (Quanterix, Billerica, MA) and the Quanterix TGF-β assay.

The detailed description of the blood sampling and storing procedure, Elisa measurement using the Quantikine (R&D Systems, Inc) and Quanterix methods is given in Supplemental Appendix S1.

Statistical analysis

On the basis of previous work from our laboratory, a difference in PWV of 50 cm/s was considered clinically

Table 1. Demographic characteristics and blood pressure analysis

	Younger than 20 years			Older than 20 years			All patients		
Characteristic	Control (n = 51)	Marfan (n = 27)	*P*	Control (n = 36)	Marfan (n = 17)	*P*	Control (n = 87)	Marfan (n = 44)	*P*
Age, years	13.6 (9.6-17.7)	14.0 (9.7-17.4)	NS	41.3 (28.8-45.6)	39.8 (29.5-48.4)	NS	18.2 (12.5-35.3)	17.9 (12.9-35.5)	NS
Sex, male:female	28:23	14:13	NS	14:13	7:10	NS	42:45	21:23	NS
Height, cm	161.3 (135.9-167.5)	173 (151-185)	0.003	169 (164.1-177.2)	180.7 (171.2-188.5)	0.001	165.8 (153.5-171.1)	176 (164-186)	< 0.001
Weight, kg	51.3 (31.3-61.6)	57.3 (40-68.7)	NS	71.0 (64.9-77.6)	76.4 (62.1-85.7)	NS	63.1 (42.6-76.4)	63.1 (42.6-76.4)	NS
BSA, m^2	1.55 (1.10-1.69)	1.79 (1.28-1.89)	NS	1.83 (1.69-1.96)	1.95 (1.79-2.13)	NS	1.67 (1.42-1.84)	1.83 (1.43-1.95)	0.04
BMI	20.0 (17.1-22.6)	17.4 (15.8-19.5)	0.005	24.7 (22.3-27.2)	22.1 (19.4-26.9)	NS	22.2 (19.5-25.5)	18.6 (16.6-22.4)	0.002
SBP, mm Hg	107 (100-115)	101 (97-110)	NS	113 (106-120)	115 (111-124)	NS	110 (100-116)	110 (100-115)	NS
DBP, mm Hg	60 (58-69)	63 (57-66)	NS	68 (63-74)	68 (61-74)	NS	65 (60-71)	64 (59-70)	NS
PP, mm Hg	42 (40-48)	42 (35-49)	NS	45 (38-49)	50 (48-55)	0.008	43 (39-48)	47 (36-50)	NS

Comparison of the whole cohort of MFS and LDS patients with age- and sex-matched control participants on the basis of their ages of younger or older than 20 years. The values presented are medians and (interquartile range) except where otherwise noted.

BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; LDS, Loeys-Dietz syndrome; MFS, Marfan syndrome; NS, not significant; PP, pulse pressure; SBP, systolic blood pressure.

Table 2. Measurements of the aorta

	Younger than 20 years			Older than 20 years			All patients		
	Control (n = 51)	Marfan (n = 27)	*P*	Control (n = 36)	Marfan (n = 17)	*P*	Control (n = 87)	Marfan (n = 44)	*P*
Ao annulus, cm	1.9 (1.7-2.0)	2.1 (1.8-2.3)	0.006	2.0 (1.9-2.2)	2.3 (2.1-2.3)	0.004	1.9 (1.8-2.1)	2.2 (1.9-2.3)	< 0.001
SoV, cm	2.5 (2.4-2.8)	3.5 (2.9-3.8)	< 0.001	2.9 (2.6-3.3)	3.6 (3.4-4.2)	< 0.001	2.7 (2.4-3.0)	3.6 (3.2-3.9)	< 0.001
SoV z-score	0.16 (−0.28 to 0.93)	3.0 (2.3-4.3)	< 0.001	−0.52 (−1.24 to 0.38)	1.4 (0.2-3.1)	< 0.001	−0.19 (−0.76 to 0.72)	2.7 (1.4-4.0)	< 0.001
ST junction, cm	2.1 (2.0-2.3)	2.4 (2.3-2.8)	< 0.001	2.5 (2.3-2.8)	2.8 (2.5-3.1)	0.014	2.3 (2.1-2.5)	2.6 (2.3-3.0)	< 0.001
SoV:annulus	1.33 (1.26-1.42)	1.70 (1.46-1.84)	< 0.001	1.45 (1.33-1.57)	1.70 (1.51-1.84)	< 0.001	1.36 (1.28-1.47)	1.70 (1.50-1.84)	< 0.001

Comparison of the diameters of the aortic (Ao) annulus, sinus of Valsalva (SoV), SoV z-score, sinotubular (ST) junction, and ratio of SoV:annulus in Marfan syndrome patients vs age- and sex-matched control participant on the basis of their ages of younger or older than 20 years.

significant.[19] Separate subgroup analyses were performed on younger (younger than 20 years) vs older groups (older than 20 years) as well as medication status (no-medication vs atenolol vs losartan). Free and total TGF-β measured using Quantikine (R&D Systems, Inc) were compared with TGF-β obtained using Quanterix assays. Frequency tables were generated for all categorical variables. Median (interquartile range) values are reported for all continuous variables. A Mann-Whitney *U* test was used to determine differences between 2 groups. A Kruskal-Wallis test with a post hoc pairwise Mann-Whitney *U* test was used to compare difference between more than 2 groups. A Spearman ρ was calculated to determine significant correlations between variables. A κ statistic was used to calculate the level of agreement between the 2 methods used to calculate TGF-β1. All tests were 2-sided and P values < 0.05 were considered statistically significant. All analyses were done using SPSS Version 25 (IBM Corp, Armonk, NY).

Results

Forty-four patients, 40 with MFS and 4 patients with LDS (21 male and 23 female) and 87 control participants (42 male and 45 female) were enrolled. Of these, 27 MFS and 51 control participants were younger than 20 years. Table 1 shows the complete demographic data. For ease of description, we have included the LDS patients in the category of MFS in the subsequent text and tables. As expected, the heights of the whole MFS group and MFS subgroups were higher than for control participants ($P < 0.01$). The weights and body surface areas were not different across the whole or MFS subgroups but the body mass indexes (BMIs) were higher for the control participants in the whole and younger age groups than the corresponding MFS groups ($P = 0.005$), and was similar between the older MFS and control subgroups. Thus, the older MFS subgroup showed relatively higher levels of obesity with age. The BP results show that only pulse pressure was higher in the older MFS subgroup compared with control participants ($P = 0.008$).

Aortic root measurements are shown in Table 2. As expected, the diameters of all of the aortic measures were greater in the whole MFS and MFS subgroups than in control participants ($P < 0.01$).

The results of the indexes of aortic stiffness are shown in Table 3. PWV was greater in the whole MFS cohort and younger MFS subgroup than in control participants but not in the older MFS subgroup compared with control participants ($P < 0.01$). There was no difference in the input or characteristic impedance between the MFS groups and control participants. The elastic strain modulus was greater in the MFS subgroups than in control participants ($P < 0.05$); the β-index was greater in the whole MFS cohort and younger subgroup than in control participants ($P < 0.01$), but the older MFS subgroup was not different from control participants. Thus, stiffness indexes converged in the older MFS patients compared with control participants.

Table 4 shows the results of plasma biomarkers measured using both assays. Plasma MMP-2 levels were significantly higher in patients with MFS compared with control participants ($P = 0.004$). However, there were no significant

Table 3. Measures of aortic stiffness

Measure	Younger than 20 years			Older than 20 years			All patients		
	Control (n = 51)	Marfan (n = 27)	*P*	Control (n = 36)	Marfan (n = 17)	*P*	Control (n = 87)	Marfan (n = 44)	*P*
PWV, cm/s	375 (328-425)	439 (391-542)	< 0.001	454 (423-491)	500 (397-608)	NS	409 (344-455)	450 (393-576)	0.003
Zi	200 (171-232)	194 (158-252)	NS	187 (142-208)	178 (155-220)	NS	192 (162-221)	184 (158-231)	NS
Zc	140 (121-175)	136 (113-195)	NS	152 (130-176)	130 (114-164)	NS	144 (122-175)	136 (113-169)	NS
Ep	253 (210-315)	338 (262-488)	0.003	455 (325-529)	560 (418-899)	0.04	314 (235-444)	429 (302-650)	0.002
β Index	2.31 (2.13-2.45)	2.60 (2.36-2.98)	< 0.001	2.82 (2.57-2.97)	2.89 (2.68-3.36)	NS	2.45 (2.22-2.81)	2.77 (2.47-3.33)	0.001

Comparison of the biophysical properties of the aorta in Marfan syndrome patients vs age- and sex-matched control participants on the basis of age of younger or older than 20 years old.
Ep, strain modulus; NS, not significant; PWV, pulse wave velocity; Zc, characteristic impedance; Zi, input impedance.

differences in plasma MMP-9 levels, or free or total TGF-β1 levels in MFS vs control participants. There was a high level of agreement between the 2 methods used to measure total TGF-β1 ($P < 0.001$; Table 5). The subgroup analysis was predicated on the results of the comparison of the total MFS group with the control participants and, because they were so similar, we believe it was not justified.

The comparison of the demographic characteristics, aortic diameters, and vascular function of the no medication subgroup or subgroups treated with either atenolol or losartan is shown in Supplemental Table S1. The no medication subgroup was older, taller, heavier, had greater body surface area and BMI than those treated with either of the medications ($P < 0.05$). The annulus, sinus of Valsalva (SoV), and sinotubular junction were not different between the groups but the SoV z-score was lower in the no medication subgroup ($P = 0.02$). There were no differences in PWV, FMD, or free or total TGF-β levels between the no medication, β-blocker and angiotensin receptor blocker (ARB) subgroups. Median FMD for younger MFS patients was 10.1 vs 6.4 for older MFS patients ($P < 0.001$). There were no significant differences in input impedance, characteristic impedance, strain modulus, β-index, systolic BP, diastolic BP, pulse pressure, or heart rate for the no medication vs β-blocker vs ARB groups, which are not shown.

The results of correlation analyses are shown in Tables 6 and 7. PWV strongly correlated with age in MFS and control participants and age-related measures of aortic diameter in control participants but less so in MFS patients. In MFS patients, FMD also correlated with age. Figure 1 shows the correlation between PWV and age in control participants and MFS patients across all age groups. Figure 2 shows subanalysis of the effect of age on FMD in MFS patients across all age groups.

Discussion

Our study showed a difference in PWV between the younger MFS subgroup and control participants; age-related arterial stiffening occurs in both groups, abolishing the difference between them. The finding of decreased endothelial function is consistent with previous human and mouse model studies of MFS.[7,20] In this study we did not find that total or free TGF-β1 was increased in our MFS patients. The biomarker results showing increased MMP-2 levels but not TGF-β1 or MMP-9 are novel findings.

As expected, the heights, sinus of Valsalva and sinotubular junction dimensions were significantly larger in the MFS patient group and subgroups than in control participants. Of interest was the finding that although the BMI in MFS patients and the younger MFS subgroup was less than in control participants, the BMI in older MFS subgroup was not, suggesting a relative increase in weight.

Different methods of measuring arterial stiffening have been used in a number of studies of MFS.[19,26-28] Because the elastic aorta acts as a conduit and a cushion for pulsatile arterial flow, stiffness of the aorta is manifested by increased PWV. It is important to differentiate between studies that measured PWV directly and those that measured aortic dimensional change to derive stiffness or its reciprocal, distensibility. The latter are suboptimal because they are on

Table 4. Plasma biomarkers measurement

Biomarker	Control (n = 37)	Marfan (n = 39)	*P*
MMP-2	171.5 (154.3-193.9)	195.7 (170.1-228.8)	0.006
MMP-9	44.3 (27.9-96.4)	58.9 (42.2-83.8)	NS
TGF-β 1,2,3, pg/mL	882.2 (711.6-1166.9)	930.4 (811.3-1285.1)	NS
TGF-β (Quanterix*)	1191.2 (979.8-1455.4)	1274.5 (1060.6-1429.21)	NS
TGF-β Free	1.29 (0.47-2.17)	0.88 (0.50-1.93)	NS

Comparison of the plasma levels of MMP-2, MMP-9, and free TGF-β1 in Marfan syndrome patients vs age- and sex-matched control participants.
MMP, matrix metalloproteinase; TGF, transforming growth factor.
* Measurements obtained using a Quanterix® (Billerica, MA) Simoa HD-1 analyzer and TGF-β kit.

the basis of the stress/strain relationship. A larger aorta, as in MFS, will distend less to absorb a similar stroke volume than a normal-sized aorta and, on the basis of the formula, strain will be less and stiffness will always compute to be increased. The same criticism can be made of methods that derive PWV from aortic dimensions. Increased PWV has been shown to be the earliest marker of cardiovascular risk in adults.[29]

A limited number of studies have measured PWV directly, using magnetic resonance imaging, carotid-femoral or echocardiographic methods, and all report increased PWV in patients with MFS, implying increased stiffness.[19,26-28] This study, which has a wide age range of subjects, confirms these findings, but only in the younger patients. Despite the fact that the aortas in the older MFS subgroup were less dilated, it was interesting to find that as the MFS and control groups aged, their PWV increased and their 2 slopes converged. Age-related increases in PWV in the normal population are well reported but it is not clear why there was convergence of the slopes between MFS patients and control participants in this study. Because most patients who require surgery for aortic root dilation are in the 20 plus age group, this finding suggests that monitoring of arterial stiffness does not offer any clinical benefit in the follow-up of these patients. However, to conclusively assess this, a larger study using this technique with a number of surgical and nonsurgical patients would be needed.

To our knowledge, our study is the first to show that there was early onset and progressive decline in FMD with age in MFS patients. In the normal population, FMD has been shown to remain constant until the fourth decade in men, and the fifth decade in women after which a steady decline develops.[30] These observations are consistent with the results of human studies[20] and in the MFS mouse model,[7] which showed endothelial dysfunction.

In contrast with previous studies,[10,11] we did not find increased levels of TGF-β1, or decreased levels of MMP-9[31] among our MFS cohort. Rather we found increased levels of MMP-2. Because of the role of maladaptive TGF-β signalling in MFS and LDS, circulating levels of this centrally important cytokine have been proposed as a prognostic and therapeutic marker.[10,11] The ELISA method and the TGF-β1 assay we used was the same Quantikine immunoassay as those studies.[32-34] To confirm this result, and in keeping with the suggestion that free TGF-β1 may be a more relevant marker,[35] we analyzed free and total TGF-β1 levels using the Quanterix immunoassay. Free TGF-β1 was not increased and total TGF-β1 levels were very similar using both assays. Therefore, it is unlikely that the discrepancy between our results and those reporting increased TGF-β1 levels is rooted in the analysis itself. Instead, our results are consistent with other studies that reported no increased levels of circulating TGF-β1 in patients with MFS. A Japanese study also reported no significant difference in the mean plasma TGF-β1 level between the MFS group and control participants.[12] Those investigators suggested that circulating TGF-β1 is not a diagnostic and therapeutic marker for Japanese MFS patients, although their findings did not exclude the association of TGF-β with the pathogenesis of MFS. In contrast, another Japanese study showed that circulating TGF-β1 levels are approximately fivefold higher in patients with acute aortic dissection compared with control participants,[36] with type B dissections showing an approximately twofold elevation in circulating TGF-β1 levels compared with type A dissections. This suggested that circulating TGF-β1 levels might be a biomarker in acute aortic dissection, but not for disease status, dilation, or risk prediction in patients with MFS. A recent study reported that a number of MFS patients who had emergency surgery for dissection had smaller aortic root sizes than that quoted in the guidelines for surgery, underscoring the need for better predictors of dissection.[37]

Our findings suggest that dysregulated TGF-β signalling is not reflected in circulating free or total TGF-β concentrations. TGF-β signalling is complex and modulated by a broad number of auxiliary cascades in a tissue-, cell-, and context-specific manner. Because of the diversity of the biological processes it affects and regulates, we speculate that circulating TGF-β concentrations are generally maintained within a relatively narrow range. By extension, the perturbations in TGF-β signalling that characterize MFS and LDS might result

Table 5. Comparison of TGF-β measured using different methods

	TGF-β 1,2,3, pg/mL	TGF-β (Quanterix*)	TGF-β free
Control			
TGF-β 1,2,3, pg/mL	–	0.887†	0.822†
TGF-β (Quanterix*)	0.967†	–	0.887†
TGF-β free	0.822†	0.887†	–
Marfan			
TGF-β 1,2,3, pg/mL	–	0.970†	0.875†
TGF-β (Quanterix*)	0.970†	–	0.958†
TGF-β free	0.875†	0.958†	–

TGF, transforming growth factor.
* Measurements obtained using a Quanterix® (Billerica, MA) Simoa HD-1 analyzer and TGF-β kit.
† $P < 0.001$.

Table 6. Correlation analysis of aortic stiffness

	Younger than 20 years				Older than 20 years				All patients			
	Control		Marfan		Control		Marfan		Control		Marfan	
	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*
PWV vs age	0.26	NS (0.068)	0.47	0.013	0.49	0.002	0.44	NS (0.07)	0.59	< 0.001	0.41	0.006
PWV vs Ao Ann	0.23	NS	0.25	NS	0.27	NS	0.23	NS	0.36	0.001	0.28	NS
PWV vs SoV	0.49	0.001	0.37	0.04	0.27	NS	−0.17	NS	0.55	< 0.001	0.20	NS
PWV vs SoV z-score	0.33	0.033	0.14	NS	−0.03	NS	−0.40	NS	0.06	NS	−0.28	NS
PWV vs SoV:Ann	0.31	0.04	0.22	NS	0.03	NS	−0.41	NS	0.34	0.002	−0.02	NS
PWV vs sinotubular junction	0.39	0.008	0.35	NS	0.46	0.005	0.01	NS	0.64	< 0.001	0.30	0.049

Correlation among pulse wave velocity (PWV) and biophysical properties of the aorta in Marfan syndrome patients vs age- and sex-matched control participants. Groups separated according to age younger or older than 20 years (Spearman ρ calculated).

Ann, annulus; Ao, aortic; NS, not significant; SoV, sinus of Valsalva; sinotubular junction, sinotubular junction.

from compensatory and/or parallel signalling cascades within the context of the vascular microenvironment. Indeed, our findings might support the notion that there is an interplay of complex processes that yield the vascular phenotype of MFS.

Our finding of endothelial dysfunction suggests accelerated ageing in those with MFS. In an MFS mouse model, mesenteric arterial endothelium-dependent relaxation stimulated by acetylcholine was significantly decreased, suggesting an impairment of nitric oxide release.[38] Endothelial vasoactive mediators regulate smooth muscle contractility and vascular smooth muscle tone.[39-42] Decreased endothelial function in those with MFS is also associated with increased plasma levels of homocysteine,[43,44] and can be compromised through increased arterial wall stiffness.[45] Nitric oxide bioavailability is decreased due to aging.

To our knowledge, the current study is the first to examine plasma levels of MMP-2 and MMP-9 in patients with MFS and those with LDS. Our findings of increased MMP-2 is consistent with increased expression of MMPs, which has been observed in human abdominal aneurysm tissue.[46,47] MMPs constitute a tightly regulated family of zinc-dependent endopeptidases that proteolytically cleave most components of the base membrane and the extracellular matrix. They play an important role in the regulation of a variety of physiological processes, including angiogenesis and vascular remodelling, and are also involved in vascular diseases such as hypertension, atherosclerosis, and aortic aneurysm. Increased MMP activity is accompanied by a widespread deterioration of microfibrils, and elastic and collagenous fibres, which eventually leads to the loss of extracellular matrix integrity, endothelial dysfunction, and reduction of smooth muscle contractility.[48] Our previous studies showed that progression of aortic aneurysm in a mouse model of MFS is associated with upregulation of MMP-2 and MMP-9.[6,7] Interestingly, in a recent study it was reported that serum MMP-2 levels correlated positively, whereas MMP-9 levels did not correlate and showed greater variation in patients with aortic root/ascending aortic aneurysms.[49] In the mouse model of MFS, we have previously shown that doxycycline, an antibiotic and a general inhibitor of MMPs, could correct aortic elastin fibre structure and organization and was more effective than atenolol in preventing thoracic aortic aneurysm.[50,51] Another study in our laboratory reported that drug therapy with losartan and doxycycline completely suppressed aneurysm formation in MFS mice, improved elastic fibre organization, downregulated MMP-2/-9 and TGF-β1 expression, and normalized aortic contractile and relaxation function to control values.[52] Although the implication of the findings in this study is to treat these patients with MMP inhibitors, the unsuccessful results of clinical trials of ARBs have shown that there needs to be caution in extrapolating animal model results to humans.[53,54]

The comparison of the results of patients not receiving medications with those receiving either a β-blocker or ARB is complicated by the fact that the no medication subgroup was older and had relatively smaller SoV z-scores, an approach consistent with recent treatment guidelines.[55] Of note, there was no difference in the PWV or FMD between the 3 groups. Of interest also is that there was no difference in free or total TGF-β levels between the 3 groups.

Limitations

The results of this study might have been affected by the total number of patients studied, which is relatively small compared with major therapeutic trials, especially in the number of older MFS patients. We recognize that our study might not have been sufficiently powered to detect differences in TGF-β1. Because PWV increases with age in the normal population, it would have been preferable to have more older patients and control participants to further parse out the relationship between PWV and age. A larger number of patients might have also shed more information about the loss of endothelial function and the role of MMP-2 in determining the aortic size and vascular function.

Table 7. Correlation analysis of FMD

FMD vs	Marfan (n = 37)	*P*
Age	−0.50	0.002
Height	−0.21	NS
Weight	−0.42	0.011
BSA	−0.36	0.03
BMI	−0.43	0.008
PWV	−0.29	NS (0.08)
SoV z-score	0.333	0.046

Correlation among flow-mediated dilation (FMD) and demographic parameters, pulse wave velocity (PWV), and sinus of Valsalva (SoV) z-score in Marfan syndrome patients.

BMI, body mass index; BSA, body surface area.

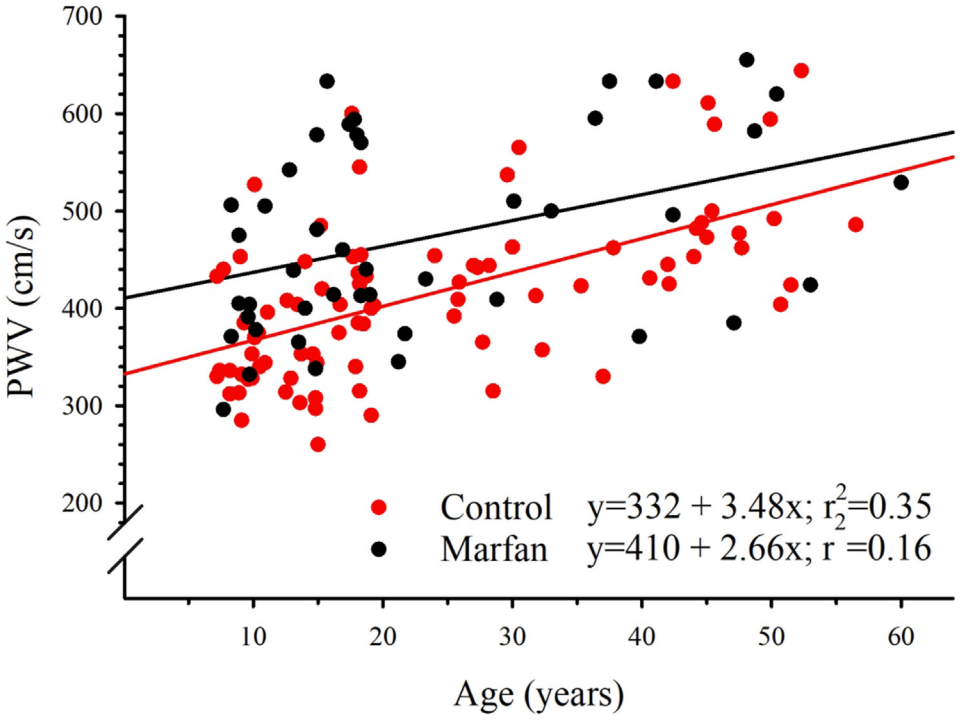

Figure 1. Correlation of pulse wave velocity (PWV) vs age in control participants and Marfan patients across all age groups.

Conclusions

In MFS patients, PWV was increased and was associated with age, independently of aortic root size. We showed premature age-related decline in FMD in MFS patients. Our study confirms the elevation of serum MMP-2 in MFS patients but not MMP-9, and total or free TGF-β1. The clinical implications are that: (1) routine evaluation of aortic stiffness or serum TGF-β1 do not help in monitoring MFS; and (2) therapies that inhibit MMP-2 with or without treatment to improve endothelial function warrant evaluation in patients with MFS.

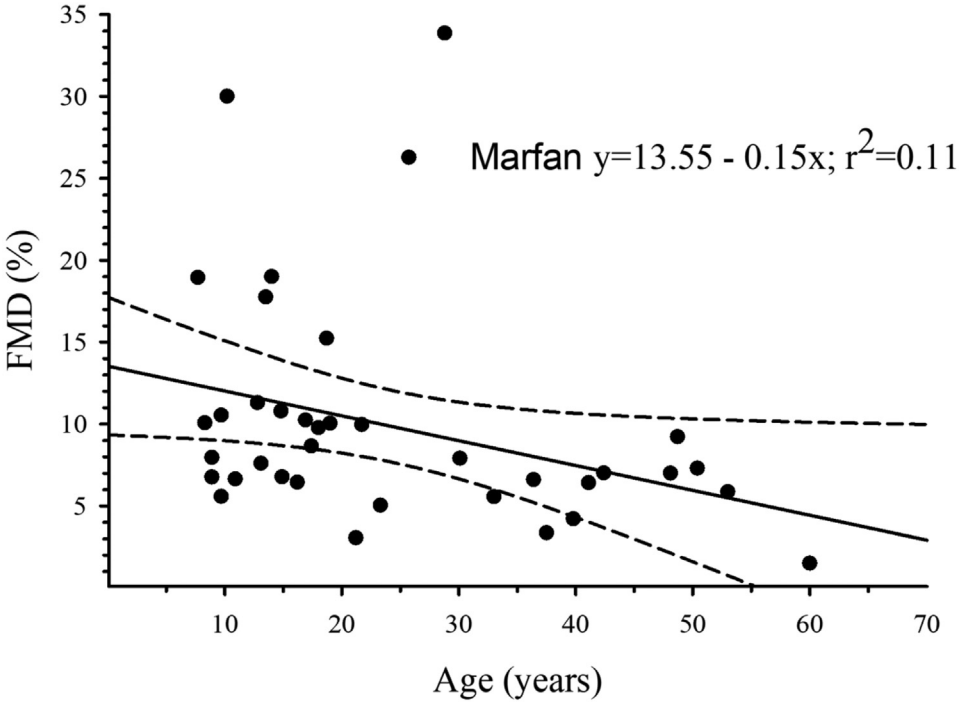

Figure 2. Correlation of flow-mediated dilation (FMD) vs age in Marfan syndrome patients across all age groups.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

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