Marfan syndrome: Getting to the root of the problem

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Adapted from:
Increased aortic tortuosity indicates a more severe aortic phenotype in adults with Marfan syndrome

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Abstract

**Background:** Patients with Marfan syndrome (MFS) have a highly variable occurrence of aortic complications. Aortic tortuosity is often present in MFS and may help to identify patients at risk for aortic complications.

**Methods:** 3D-visualization of the total aorta by MR imaging was performed in 211 adult MFS patients (28% with prior aortic root replacement) and 20 controls. A method to assess aortic tortuosity (aortic tortuosity index: ATI) was developed and reproducibility was tested. The relation between ATI and age, body size and aortic dimensions at baseline was investigated. Relations between ATI at baseline and the occurrence of a clinical endpoint (aortic dissection, and/or aortic surgery) and aortic dilation rate during 3 years of follow-up were investigated.

**Results:** ATI intra- and interobserver agreement were excellent (ICC: 0.968 and 0.955, respectively). Mean ATI was higher in 28 age-matched MFS patients than in the controls (1.92±0.2 versus 1.82±0.1, p=0.048). In the total MFS cohort, mean ATI was 1.87±0.20, and correlated with age (r=0.281, p<0.001), aortic root diameter (r=0.223, p=0.006), and aortic volume expansion rate (r=0.177, p=0.026). After 49.3±8.8 months follow-up, 33 patients met the combined clinical endpoint (7 dissections) with a significantly higher ATI at baseline than patients without endpoint (1.98±0.2 versus 1.86±0.2, p=0.002). Patients with an ATI>1.95 had a 12.8 times higher probability of meeting the combined endpoint (log rank-test, p<0.001) and a 12.1 times higher probability of developing an aortic dissection (log rank-test, p=0.003) compared to patients with an ATI<1.95.

**Conclusions:** Increased ATI is associated with a more severe aortic phenotype in MFS patients.
Introduction

Patients with Marfan syndrome (MFS) are at risk for potentially fatal aortic dissections.\textsuperscript{1,2} Aortic dissection is generally preceded by aortic dilation, which is regularly monitored by aortic imaging in clinical practice. At a certain threshold diameter, prophylactic aortic surgery is advised by current clinical guidelines.\textsuperscript{3,4} However, identification of MFS patients at high risk for aortic dissection remains difficult and seems to be dependent on prior aortic root surgery, aortic size, aortic distensibility and aortic diameter growth above 0.5 mm/year.\textsuperscript{5} Besides gradual expansion of the aortic diameter, the aorta also elongates.\textsuperscript{6} Considering the aorta in its anatomically fixed position, aortic elongation may force the vessel to curve and become tortuous.\textsuperscript{7} Tortuosity of smaller arteries has already been shown to be a marker for adverse cardiovascular outcomes in patients with connective tissue disorders.\textsuperscript{8,9} However, in MFS patients the aorta rather than the smaller arteries, is generally affected. Therefore, we adapted and applied the tortuosity index of smaller arteries on 3D aortic images, acquired by magnetic resonance imaging (MRI) to establish the aortic tortuosity index (ATI).\textsuperscript{8-11} We subsequently correlated ATI with aortic expansion rate and clinical endpoints in 3-4 years of follow-up.

We hypothesized that ATI may serve as a marker for severity of aortic disease and may predict aortic expansion rate and clinical outcome, such as elective aortic surgery and aortic dissection.

Methods

Study subjects

Our study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the medical ethics committee of the Academic Medical Centre. All participating subjects gave written informed consent. All patients were participants of the COMPARE study, a randomized trial in which the effect of losartan therapy (100mg, daily) on the aortic dilation rate was assessed.\textsuperscript{12} At baseline and after three years of follow-up, we examined patients’ medical history and performed MRI of the entire aorta. Clinical events were evaluated until February 2014. \textit{FBN1} mutation analysis was performed in all patients. When no \textit{FBN1} mutation was found, the following connective tissue genes were subsequently screened: \textit{TGFB1, TGFB2, TGFB2, MYH11, MYLK1, SMAD3, and ACTA2}. Because of the possible impact of mutations other than \textit{FBN1} on ATI, we excluded patients with a pathogenic non-\textit{FBN1} mutation for the main analysis. These excluded patients were separately analysed.
**Purpose and outcomes**

The primary aim of this study was to investigate the association of ATI with severity of aortic phenotype in MFS patients. Hereto, we 1) investigated applicability and reproducibility of ATI in MFS patients (n=14) by two investigators, 2) compared ATI of control subjects in whom aortic disease was definitely ruled out (n=20) with age, sex and length-matched MFS patients (n=28), 3) correlated ATI with age, aortic root diameter and aortic volume at baseline, and 4) compared ATI between MFS subgroups based on genotypes.

Secondary aims were to assess the predictive value of ATI during follow-up on aortic root dilation rate, aortic volume expansion rate, the combined clinical endpoint (prophylactic aortic surgery, aortic dissection and death), and separately on aortic dissection. The decision to perform prophylactic aortic surgery was completely at the discretion of the attending cardiologists, based on European and American guidelines.3,4

**Magnetic Resonance Imaging**

All MRI scans were obtained in two centres (AMC, Amsterdam and LUMC, Leiden, The Netherlands) with a 1.5 Tesla MR system Avanto (Siemens, Erlangen, Germany) or a Philips (Intera, release 11 and 12; Philips Medical Systems, Best, the Netherlands). Contrast-enhanced MRI of the total aorta was performed using standardised protocols, which has been described previously.12,13 In case of significantly reduced image quality due to metal scoliosis implants, patients were excluded from analysis. Aortic root diameter was assessed by measuring the greatest diameter between the three cusp-cusp dimensions in diastole. Aortic volume was measured from the aortic annulus to the aortic bifurcation, as described previously.13

**Aortic Tortuosity Index**

ATI was calculated as the ratio of ‘aortic length’ to ‘geometric length’ (Figure 1). The aortic length was defined as the length of a centreline through the entire aorta (from annulus to aortic bifurcation) created by manually placed seeding points through the lumen of the aorta in the axial, sagittal and coronal plane using vessel analysis software (3mensio vascular, 3Mensio Medical Imaging BV, Bilthoven, the Netherlands). Geometric length was considered to be the Cartesian distance between aortic valve annulus and aortic bifurcation. The measurement of ATI can be easily performed within 5 minutes.

**Statistical analysis**

Statistical analysis was performed using SPSS (SPSS, release 20.0 for Windows). Intra- and interobserver agreements for ATI were determined with the calculation of the intraclass correlation coefficients (ICCs) and were processed using Bland-Altman plots. We matched the control subjects to Marfan patients of this cohort based on age (±5 years), sex and height (±10 cm). Linear regression analysis and the Pearson’s correlation coef-
Chapter 3: Aortic tortuosity predicts aortic severity

facet were used to correlate ATI with aortic root diameter, aortic volume, body surface area, age and aortic volume expansion rate. Means of different groups were compared with the Student’s t-test or paired t-test where appropriate. Uni- and multivariate Cox-regression analysis was used to demonstrate whether ATI was an individual risk-factor for clinical outcome. Optimal cut-off point for ATI was determined by maximizing the c-statistic of the cox-model. Kaplan Meier analysis and the Log Rank test were used to compare the high and low ATI groups. To compare the patients with a TGFBR1, TGFBR2, TGFB2, MYH11 or MYLK1 mutation to patients with a FBN1 mutation, ANOVA and Mann-Whitney U tests were used.

**Results**

A total of 233 patients were included in the COMPARE trial. For the present study, we excluded patients with a non-FBN1 mutation (n=9), a distal aortic graft (n=7), a scoliosis implant which led to large artefacts on MRI (n=4), and those who underwent

Figure 1. View of image processing software. The seeding points are placed manually central in the coronal, sagittal and axial planes in the contrast enhanced lumen of the aorta (left). Aortic length (AL) is measured in a multiplanar reconstructed stretched view of the aorta. Geometric length (GL) is the Cartesian distance between its 2 endpoints calculated by exported spatial coordinates. Aortic Tortuosity Index (ATI) is measured by dividing AL (in blue) by GL (in yellow).
CT-scanning instead of MRI (n=2). Baseline ATI measurement was performed in 211 patients (Figure 2), whose characteristics are shown in Table 1.

**Aortic tortuosity index is associated with severity of aortic phenotype**

The mean difference of the intra- and interobserver variability of ATI were 0.002 ± 0.0274 (ICC: 0.968) and 0.002 ± 0.0342 (ICC: 0.955) in 14 MFS patients, respectively. In non-MFS controls (n=20), ATI was significantly correlated with age (r=0.740, p<0.001, Figure 3A). The control subjects were significantly older (52.5 years ± 12 versus 36 years ± 13, p<0.001), shorter (182 SD 8cm versus 188 SD 11cm, p=0.006) and more often male (85% versus 53%, p<0.001) compared to the total MFS cohort. Therefore, we compared ATI of the control subjects with sex-, age- and height-matched MFS patients (Table 2). Mean ATI was significantly lower in controls as compared to the matched MFS patients (1.82 SD 0.1 versus1.92 SD 0.2, p=0.048).

In the total MFS cohort (n=211), mean ATI was 1.87 SD 0.20. We found a positive correlation between ATI and age (r=0.281, p<0.001, Figure 3A) and only a trend towards a negative correlation between ATI and body surface area (r=-0.119, p=0.086). We did not find a difference in ATI between females and males (1.89 SD 0.2 versus 1.86 SD 0.2, respectively, p=0.38). We could also not demonstrate a significant difference in ATI between patients with and without prior aortic root replacement at baseline (1.88 SD 0.2 versus 1.87 SD 0.2, p=0.73, Table 1). Finally, ATI correlated both with diameter of the aortic root (r=0.223, p=0.006) and volume of the total aorta (r=0.280, p<0.001, Figure 3B).

All patients in our cohort were diagnosed with MFS based on the Ghent criteria of 1996. Patients also fulfilled the revised Ghent criteria of 2010, and did not have distinguishing features of other connective tissue disorders. Patients without a *FBN1* mutation (n=34, 15%) did not differ significantly in ATI from the MFS patients with a known *FBN1* mutation (n=177, 84%) 1.86 SD 0.15 versus 1.88 SD 0.22, respectively, p=0.54. Interestingly, the ATI of the *TGFBR2* patients (n=2) seemed higher as compared to non-mutation MFS patients (ATI=2.21 SD 0.10 versus 1.86 SD 0.15, p=0.018) and slightly higher as compared to *FBN1* mutation MFS patients (p=0.13, Figure 4). The *TGFBR2* patients were comparable to the total cohort without distinguishing features of Loeys-Dietz syndrome (LDS). One had a height of 181 cm and an age of 25 years, and one patient had a height of 185 cm and 58 years. ATI of patients with mutations in other connective tissue genes than *FBN1* or *TGFBR2* was similar to that of the *FBN1* patients (Figure 4).
Aortic tortuosity index predicts aortic disease progression during follow-up

After three years of follow-up, mean ATI increased slowly but not significantly in MFS patients (+0.02 SD 0.1, p=0.12). Baseline ATI measurements were significantly associated with aortic volume expansion rate in three years follow-up (r=0.177, p<0.001), but not with aortic root dilation rate (r=0.043, p=0.63). After a mean follow-up of 49.3 SD 8.8 months from the first MRI scan, clinical endpoints were reached in 33 patients: 26 elective aortic root replacements and 7 type B aortic dissections (no deaths or type A aortic dissections).

We also assessed the effects of losartan treatment (100mg, daily) on the ATI. There was no difference in baseline ATI between patients receiving losartan or no additional losartan therapy (1.87 SD 0.2 versus 1.88 SD 0.2, p=0.55). Losartan therapy did not influence the occurrence of the combined clinical endpoint (16 versus 17, p=0.46) or the

![Figure 3](image)

**Figure 3.** Scatterplots demonstrating an association of aortic tortuosity index with A) age in Marfan patients and controls and B) aortic volume.

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients n = 211</th>
<th>Native aortic valve n = 153</th>
<th>Aortic valve replacement n = 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37 ± 13</td>
<td>36 ± 13</td>
<td>39 ± 12</td>
</tr>
<tr>
<td>Female gender</td>
<td>100 (47)</td>
<td>79 (52)</td>
<td>21 (36)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>188 ± 11</td>
<td>187 ± 11</td>
<td>190 ± 10</td>
</tr>
<tr>
<td>FBN1 mutation*</td>
<td>177 (84)</td>
<td>131 (86)</td>
<td>46 (79)</td>
</tr>
<tr>
<td>Ectopia Lentis</td>
<td>101 (48)</td>
<td>73 (48)</td>
<td>28 (48)</td>
</tr>
<tr>
<td>System score &gt; 7</td>
<td>124 (59)</td>
<td>88 (58)</td>
<td>36 (62)</td>
</tr>
<tr>
<td>Aortic root (mm)</td>
<td>44.3 ± 5</td>
<td>44.3 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>Aortic volume</td>
<td>231 ± 63</td>
<td>218 ± 53#</td>
<td>268 ± 73#</td>
</tr>
<tr>
<td>Aortic volume expansion rate</td>
<td>12 ± 63</td>
<td>10 ± 16#</td>
<td>19 ± 17#</td>
</tr>
<tr>
<td>Aortic Tortuosity Index</td>
<td>1.87 ± 0.2</td>
<td>1.88 ± 0.2</td>
<td>1.87 ± 0.2</td>
</tr>
<tr>
<td>Losartan at inclusion</td>
<td>107 (51)</td>
<td>81 (53)</td>
<td>26 (45)</td>
</tr>
<tr>
<td>β-blockers at inclusion</td>
<td>150 (71)</td>
<td>113 (74)</td>
<td>37 (64)</td>
</tr>
</tbody>
</table>

Data presented as number (percentage) or mean ± standard deviation.

* 1 patient refused genotyping # p<0.001
occurrence of aortic dissection compared to patients without losartan therapy (3 versus 4, p=0.48).

A significantly higher ATI at baseline was found in patients who met the combined clinical endpoint (1.98 SD 0.2 versus 1.86 SD 0.2, p=0.002), as well as in patients with aortic dissection (2.03 SD 0.1 versus 1.86 SD 0.2, p=0.015) compared with patients without a clinical endpoint. Univariate analysis of sex, age, BSA and aortic root dimensions as predictors for the combined clinical endpoint and aortic dissection are described in Table 3. After multiple regression analysis, ATI and aortic root diameter were the sole independent predictors for the combined clinical endpoint (Table 3, ATI: Hazard Ration (HR)=12.8, p=0.030; aortic root diameter: HR=1.451, p<0.001). ATI was the only predictor for aortic dissection (HR=12.083, p=0.039). Based on the c-statistic, an ATI value of 1.95 had largest discriminating power for aortic dissection (c-statistic of 0.75 (95%CI = 0.64 to 0.85, p=0.025). For detailed analysis of the effect of ATI on the combined endpoint and on aortic dissections, the total cohort was divided into two groups (Group 1: ATI<1.95, Group 2: ATI>1.95). Between the two groups, there was no significant difference in percentage of patients using losartan treatment (ATI<1.95: 47% versus ATI>1.95: 56%, p=0.26). Kaplan-Meier curve analysis revealed that patients with an ATI above 1.95

<table>
<thead>
<tr>
<th>Variables</th>
<th>Marfan patients N=28</th>
<th>Control subjects N=20</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 ± 9</td>
<td>52 ± 12</td>
<td>0.65</td>
</tr>
<tr>
<td>Male gender</td>
<td>19 (68)</td>
<td>17 (85)</td>
<td>0.31</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>189 ± 11</td>
<td>184 ± 10</td>
<td>0.56</td>
</tr>
<tr>
<td>Aortic length (mm)</td>
<td>528 ± 39</td>
<td>473 ± 29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Geometric length (mm)</td>
<td>277 ± 28</td>
<td>257 ± 20</td>
<td>0.005</td>
</tr>
<tr>
<td>Aortic Tortuosity Index</td>
<td>1.92 ± 0.2</td>
<td>1.82 ± 0.1</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Data presented as number (percentage) or mean ± standard deviation. HR: Hazard Ratio

Figure 4. Bar chart demonstrating increased ATI in aneurysm patients with a TGFBR2 mutation compared to Marfan patients which fulfilled the Ghent criteria of 2010 without a mutation.
had a significant higher probability of meeting the combined endpoint (log rank-test, \( p<0.001 \), Figure 5A), and a significant higher probability of experiencing a type B aortic dissection (log rank-test, \( p=0.003 \), Figure 5B).

Subgroup analysis revealed that patients with a native aortic root at the start of the study and an ATI above 1.95 had a significantly higher probability of meeting the combined endpoint (log rank-test, \( p=0.004 \)).

Table 3. Univariate and multivariate analysis for combined clinical endpoint and aortic dissection

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate endpoint (n=33)</th>
<th>Multivariate endpoint (n=33)</th>
<th>Univariate endpoint (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>P-value</td>
<td>HR</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.004</td>
<td>0.80</td>
<td>1.004</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.505</td>
<td>0.26</td>
<td>2.226</td>
</tr>
<tr>
<td>BSA (m2)</td>
<td>3.166</td>
<td>0.14</td>
<td>5.740</td>
</tr>
<tr>
<td>Aortic root diameter (mm)</td>
<td>1.454</td>
<td>&lt;0.001</td>
<td>1.451</td>
</tr>
<tr>
<td>Aortic volume (ml)</td>
<td>1.005</td>
<td>0.077</td>
<td>0.993</td>
</tr>
<tr>
<td>Aortic Tortuosity Index</td>
<td>7.174</td>
<td>0.001</td>
<td>12.841</td>
</tr>
<tr>
<td>Losartan at inclusion</td>
<td>0.860</td>
<td>0.67</td>
<td>0.658</td>
</tr>
</tbody>
</table>

Figure 5. Kaplan-Meier curve analysis demonstrated that an aortic tortuosity index above 1.95 predicts the combined clinical endpoint (A) and aortic dissection (B) in patients with Marfan syndrome.

Discussion

In this study we introduced ATI as a novel and straightforward method to measure aortic tortuosity with excellent reproducibility. ATI seemed to be comparable in males and females, was relatively independent of body stature and slowly increased over time. Several associations with aortic disease severity in adult patients with MFS were found: ATI showed positive correlations with age, aortic diameter, and aortic volume expansion.
rate. Moreover, we were able to show that a high ATI is an independent predictor of clinical events, including aortic dissection.

**Tortuosity in connective tissue disease**

The aorta dilates slowly during ageing and becomes more tortuous, which has been shown in humans without connective tissue disease.\(^6,14\) Indeed, we observed a correlation between ATI and age in our non-MFS controls, and this also holds true for the MFS patients. Importantly however, ATI was significantly higher in the MFS patients compared to the controls. Arterial tortuosity is a relatively common feature in several types of connective tissue disease, especially in Aortic Tortuosity Syndrome patients (\(SLC2A10\) mutations) and Loeys-Dietz Syndrome patients (\(TGFBR1\) and \(TGFBR2\) mutations).\(^15\) Loeys-Dietz Syndrome patients are known to have an aggressive form of aortic disease, with increased tortuosity of large and smaller arteries, and aortic dissections at an earlier age when compared to patients with a \(FBN1\) mutation.\(^15,16\) In line with these data, only the two patients with a mutation in the \(TGFBR2\) gene in our cohort, showed an excessive ATI. In our earlier work, we demonstrated increased plasma TGFβ levels and correlations between TGFβ levels and aortic disease severity in MFS, which may be related to aortic tortuosity.\(^17,18\) In line with our current and previous results, Hillebrand and colleagues also demonstrated increased levels of TGFβ in MFS and LDS, yet lower levels in patients with \(MYH11\) mutations.\(^19\) Therefore, TGFβ-mediated signalling may play a role in the development of aortic tortuosity. Tortuosity of smaller arteries in patients with MFS and LDS has already been shown to be associated with younger age at surgery, earlier age at dissection and death by Morris et al.\(^8\) Interestingly, a family with a \(TGFB2\) mutation (some specialists propose to name this syndrome ‘Loeys-Dietz Syndrome type IV’) showed many non-cardiac features related to the mutation, and tortuosity only of the smaller arteries (not of the aorta). In this family there was no history of aortic complications.\(^20\) This suggests that tortuosity may be (arterial) site specific, depending on the type of mutation, and that tortuosity of the aorta is a better predictor for aortic complications than tortuosity in other smaller arteries.

**Effect of aortic tortuosity on hemodynamic indices**

Aortic tortuosity may also contribute to aortic disease. In 1990, Wenn and Newman hypothesized that the haemodynamic effect of tortuosity creates flow profile asymmetries leading to abnormal wall shear stress patterns.\(^11\) Indeed, it has been observed that age-related prolongation of the ascending aorta is related to increased proximal aortic stiffness, even in individuals without cardiovascular disease.\(^21\) Thus, increased aortic stiffness, known to be an independent predictor for progressive aortic dilation,\(^22-24\) may also be related to aortic tortuosity in MFS patients. In our cohort, ATI did not significantly increase over time, probably due to the relatively short follow-up. However, when cor-
relating baseline ATI to aortic growth during three years of follow-up, we demonstrated that a higher baseline ATI was associated with a higher aortic volume expansion rate. On the other hand, ATI did not correlate with aortic root dilation rate. It could therefore be speculated that the altered flow profile through a tortuous aorta independently leads to a more severe (distal) aortic phenotype and enhanced susceptibility to aortic dissections.

**Clinical value of the aortic tortuosity index**

Acute aortic dissection is the most feared and potentially lethal consequence of thoracic aortic aneurysms, mostly preceded by an excessive increase in aortic diameter. However, type B aortic dissections occur frequently without significant aortic dilation in MFS, and therefore prediction of type B aortic dissections remains difficult. Besides predicting aortic volume expansion rate, we also showed that baseline ATI could be used as a prognostic tool to distinguish patients at higher risk for aortic complications. In this study, MFS patients with an ATI above 1.95 had a 12-fold increased risk for aortic dissection. ATI was actually the only significant predictor for dissection in the present cohort of MFS patients. We recently proposed a prediction model for type B dissections in MFS patients, which is composed of different variables. The prediction model showed that MFS patients with prior prophylactic aortic surgery and a slightly dilated proximal descending aorta diameter $\geq$27mm are at substantial risk for type B aortic dissection, and that losartan seemed to reduce the risk of aortic dissection. Addition of ATI may further improve the prediction accuracy of this model. We recommend measuring the ATI at least once in MFS patients, in order to discriminate patients at risk for aortic dissection.

In conclusion, ATI is a straightforward method to measure aortic tortuosity and ATI could predict MFS patients at risk for clinical events during four year follow-up. In particular, ATI independently predicted aortic dissections, which should be further evaluated in larger prospective studies in MFS patients and other aortic aneurysm patients.

**Acknowledgments**

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## References


