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Novel biomarkers associated with thoracic aortic disease

Carlijn G.E. Thijssen [a,b], Silvy Dekker [a], Lidia R. Bons [a], Laurie W. Geenen [a], Arjen L. Gökalp [c], Johanna J.M. Takkenberg [c], Mostafa M. Mokhles [c,d], Jos A. Bekkers [c], Eric Boersma [a], Elke Bouwens [a,e], Roland R.J. van Kimmenade [b,a], Jolien W. Roos-Hesselink [a,*]

[a] Department of Cardiology, Erasmus MC, Rotterdam, the Netherlands
[b] Department of Cardiology, Radboud University Medical Center, Nijmegen, the Netherlands
[c] Department of Cardiothoracic Surgery, Erasmus MC, Rotterdam, the Netherlands
[d] Department of Cardiothoracic Surgery, University Medical Center Utrecht, Utrecht, the Netherlands
[e] Department of Anesthesiology, Erasmus MC, Rotterdam, the Netherlands

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ABSTRACT

Background: Biomarkers might help to improve diagnosis, surveillance and risk stratification of thoracic aortic disease (TAD). We explored the association between a broad spectrum of cardiovascular biomarkers with clinical characteristics and thoracic aortic diameter in TAD patients.

Methods: Venous blood-samples were obtained in 158 clinically stable TAD patients visiting our outpatient clinic (2017–2020). TAD was defined as a thoracic aortic diameter $\geq$ 40 mm, or genetic confirmation (hereditary TAD). The cardiovascular panel III of the Olink multiplex platform was used for batch analysis of 92 proteins. A comparison was made between biomarker levels in patients with and without previous aortic dissection and/or surgery, and with and without hereditary TAD. Linear regression analyses were applied to identify (relative, normalized) biomarker concentrations associated with the absolute thoracic aortic diameter (AD_{max}), and thoracic aortic diameter indexed for body surface area (ID_{max}).

Results: Median age of study patients was 61.0 (IQR 50.3–68.8) years, 37.3% females. Mean AD_{max} and ID_{max} were 43.3 $\pm$ 5.4 mm and 21.3 $\pm$ 3.3 mm/m^2. After multivariable adjustment, Matrix Metalloproteinase-3 (MMP-3) and Insulin-like growth factor binding protein 2 (IGFBP-2) showed a significant positive association with AD_{max} and ID_{max}, respectively. Patients with previous aortic surgery/dissection had higher N-terminal-pro hormone BNP (NTproBNP) (median 3.67 [IQR 3.01–3.99] vs 2.84 [2.32–3.26], $p \leq 0.001$). Patients with hereditary TAD had higher Trem-like transcript protein 2 (TLT-2) (median 4.64 [IQR 4.45–4.84]) than those with non-hereditary TAD (4.40 [4.17–4.64]; $p = 0.00042$).

Conclusions: Among a broad range of biomarkers, MMP-3 and IGFBP-2 were associated with disease severity in TAD patients. The pathophysiological pathways uncovered by these biomarkers, and their potential clinical use warrants further research.

1. Introduction

Thoracic aortic disease (TAD) including thoracic aortic aneurysm and thoracic aortic dissection have an estimated incidence of 9/100,000 per year in females, and 16/100,000 per year in males [1]. An inherited pattern of TAD is found in about 20% of cases, also referred to as hereditary thoracic aortic disease [2]. Patients with (hereditary) TAD are at risk of thoracic aortic dissection, which has a high mortality and morbidity [3,4]. To prevent aortic dissection and sudden death, timely intervention is warranted. Currently, the timing of preventive surgery for TAD patients is almost solely based on the aortic diameter, since this has been associated with the risk of acute thoracic aortic dissection [5]. However, most aortic dissections occur at aortic diameters below the threshold for elective aortic surgery. Therefore, there might be an important role for other predictors of events such as blood biomarkers to improve the risk prediction of aortic dissection [6]. Biomarkers can have potential diagnostic and prognostic value relevant for minimally invasive follow-up assessment and clinical decision making in TAD patients. Ideally, biomarkers could be used to provide a more precise estimate of the risk of aortic complications for individual patients, leading to

* Corresponding author at: Erasmus MC, Rg-419, Dr. Molewaterplein 40, 3015 GD Rotterdam, the Netherlands.
E-mail address: j.roos@erasmusmc.nl (J.W. Roos-Hesselink).

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improved and more personalized treatment strategies.

Currently, no biomarkers have been found that can accurately predict the presence, severity or prognosis of TAD [7]. Several circulating markers have been investigated for diagnosis and localization of TAD, among which mediators of collagen and elastin degradation such as matrix metalloproteinases (MMP's). Overall, finding a disease specific biomarker for TAD is not easy, since many biomarkers are not very specific for TAD. There is a need for more evidence on potential biomarkers for the surveillance and prognosis of TAD.

Recently, the proximity extension assay, has enabled the screening of 92 proteins simultaneously with high sensitivity and specificity in small biological sample volumes [8]. This technique has already been investigated in patients with abdominal aortic aneurysm (AAA), were plasma levels of 21 proteins were found to be significantly different in patients with AAA compared to controls [9]. However, different biomarkers might be associated with TAD, since TAD has a different etiology than AAA and is less strongly associated with risk factors of atherosclerosis [10].

With the use of this technology, we aimed to identify biomarkers associated with clinical characteristics and thoracic aortic diameter in patients with TAD, which is the first step in finding biomarkers for clinical use in diagnosis, surveillance and prognosis of TAD.

2. Methods

2.1. Study population

All consecutive patients who visited the specialized TAD outpatient clinic of our center between October 2017 and January 2020, were eligible for inclusion. A flowchart of patient inclusion is shown in Fig. 1. The TAD outpatient clinic is a specialized outpatient clinic in which patients are seen by a cardiologist and/or specialized physician assistant for cardiovascular family screening or TAD surveillance, including referral from primary care and secondary care. Our tertiary care center is a referral center for patients with (suspected) hereditary thoracic aortic disease, especially Loeys-Dietz syndrome. Inclusion criteria for our study were: adult age ($\geq$ 18 years) and thoracic aortic diameter $\geq$ 40 mm and/or a genetically confirmed hereditary TAD (with normal or abnormal aortic diameter). All eligible patients were invited to participate in this prospective cross-sectional study. For comparison of clinical characteristics, all TAD patients were divided into patients with and without previous aortic surgery and/or dissection. A second comparison was made between patients with and without hereditary TAD. This study was approved by the local ethics committee (METC Erasmus MC, MEC-2017-057), and was designed, performed and controlled in accordance with current local and international good clinical practice guidelines. Written and signed informed consent was obtained from all participants.

2.2. Data collection

All patients underwent standard care at the outpatient clinic by their treating physician. Additional data was collected from the hospitals patient files using a standardized case report form, and was documented using a secured web-based application (GEneric Medical Survey Tracker, Erasmus MC and Equipe Zorgbedrijven, latest release 2019, version 1.8.6, open source). Body Surface Area (BSA) was calculated using the DuBois and DuBois formula [11]. Indexed thoracic aortic diameter was calculated by dividing absolute thoracic aortic diameter by BSA for each individual patient.

2.3. Biomarker measurement

Fasting venous blood (6 ml) was collected in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were centrifuged and the plasma was then aliquoted and stored at −80 °C within two hours after withdrawal, until batch analysis was performed. The cardiovascular panel III of the Olink Multiplex platform (Olink Proteomics AB, Uppsala, Sweden) was used for a batch-wise analysis. This panel was selected for its well-balanced inclusion of proteins with already established associations with cardiovascular disease and abdominal aortic aneurysm, and because it has a high performance among other Olink panels. The panel is a high-throughput, multiplex immunoassay enabling simultaneous quantification of 92 CVD-related proteins by Proximity Extension Assay (PEA) technology [12]. The assay uses two oligonucleotide-labelled antibodies to bind to their respective target proteins in the sample. When the two antibodies are in close proximity, a new polymerase chain reaction target sequence is formed. The resulting sequence is detected and quantified using standard real-time polymerase chain reaction testing (PCR).

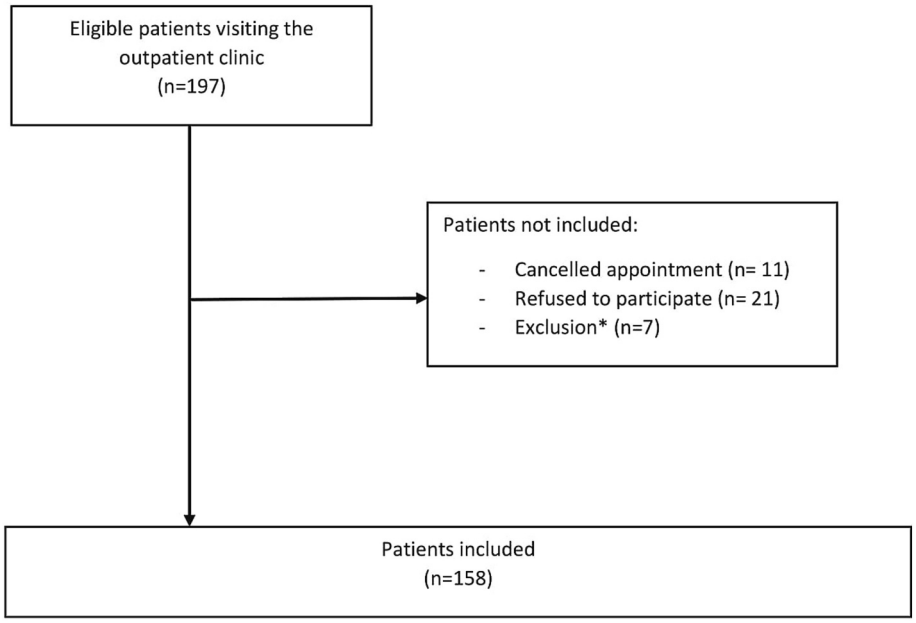

Fig. 1. Flowchart of patient inclusion.

The proteins/biomarkers are presented as Normalized Protein Expression (NPX) units, which are relative units that result from the polymerase chain reaction. The NPX units are expressed on a log2 scale. This arbitrary unit can thus be used for relative quantification of proteins and a comparison of (two)fold changes between groups. We analyzed the NPX units as standardized *Z*-scores, which enables direct comparisons of the strength of the associations between the 92 biomarkers and the study endpoints.

2.4. Imaging

The endpoints of this study were maximal absolute thoracic aortic diameter (AD_{max}) and maximal indexed thoracic aortic diameter (ID_{max}). The maximal thoracic aortic diameter was obtained using contrast enhanced and electrocardiography gated Computed Tomography imaging (CT). Thoracic aortic measurements were performed by a radiologist of the Erasmus Medical Center using a standardized protocol. The radiologist was blinded to the biomarker results. Imaging was performed on the same day the blood samples were obtained. Diameters of the Sinuses of Valsalva were measured from the cusp to commissure, ascending aorta and descending aorta were measured in two directions at the level of the pulmonary bifurcation using the double-oblique method in a reconstruction, perpendicular to the vessel axis. Additionally, the largest ascending and descending aortic diameters were measured (if not at any standardized location). The largest diameter of the measurements was used for the analysis. If no CT scan was performed on the date of inclusion, transthoracic echocardiography (TTE) measurements were used. On TTE measurements were performed using the parasternal long axis view during late diastole with the leading edge to leading edge method. CT imaging was not available in 52 patients (32.9%). Indexed aortic diameter was calculated by dividing each patients' absolute maximal thoracic aortic diameter by their body surface area (BSA).

2.5. Statistical analysis

Baseline characteristics were studied and described in relation to sex. Biomarker levels were studied in relation to TAD type (hereditary versus non) and TAD history (aortic dissection or surgery versus non). The normal distribution of continuous variables was assessed using the Shapiro-Wilk test. Students *t*-tests were used to evaluate between group differences in normally distributed continuous data which are presented as mean ± standard deviation (SD). The Mann-Whitney *U* test was used to study between group differences in skewed continuous data, which are presented as median and interquartile range (IQR). For categorical data, Chi-square test or Fisher exact test were used, and the data were presented as percentages or frequencies. The statistical significance level of the statistical tests on baseline characteristics was set at $p < 0.05$.

Linear regression analyses were performed to study the association between the biomarkers on the selected multiplex-assay and the study endpoints (AD_{max} and ID_{max}). Sub-analyses were performed to study the association between the biomarkers and ascending or descending aortic diameter separately. Stratified analyses were performed in patients with connective tissue disease and patients with a history of thoracic aortic dissection. We performed crude, unadjusted analyses, and analyses with adjustment for the potential confounders age and sex, with additional analyses for previous aortic dissection and previous aortic surgery. These analyses were performed for the total population, and stratified analyses were performed for males and females. This is an exploratory analysis of a multiplex-assay with 92 biomarkers. We therefore adjusted for inflation of the type I error due to multiple statistical testing by applying Bonferroni's correction, and the statistical significance level was set at $p < 0.00055$ for univariable analysis. Since only four biomarkers were selected for further analysis the statistical significance level was set at $p < 0.05$ for multivariable analysis.

The data-analysis was performed with statistical and computing programme *R* (R Foundation for Statistical Computing, Vienna, Austria. Version 3.6.1).

3. Results

In total, 99 males and 59 females with TAD were included, with a median age of 61.0 (50.3–68.8) years (Table 1). The biomarker SPON1 was excluded from analysis because 98% of the measurements were below the limit of detection. The remaining 91 biomarkers were included in the analyses. Ascending aortic diameter was obtained in 150 patients (95%), whereas descending aortic diameter was obtained in 104 patients (66%). Left ventricular ejection fraction (LVEF) was normal or only slightly reduced in 101 patients (63.9%), and moderately or severely reduced in 2 patients (1.2%).

Figs. 2a and 2b show the association between the selected biomarkers and the study endpoints. Matrix Metalloproteinase-3 (MMP-3; strongest association) and Chitinase-3-like protein 1 (CHI3L1) showed a significant positive association with AD_{max}, but not with ID_{max}. One SD difference in ^{2}log(CHI3L1) and ^{2}log(MMP-3) was associated with a mean difference of 1.74 mm (95% CI 0.92–2.56) and 1.48 mm (95% CI 0.66–2.30) in AD_{max}, respectively. Insulin-like growth factor binding protein 2 (IGFBP-2) and metalloproteinase inhibitor 4 (TIMP4) showed a significant positive association with ID_{max}, but not AD_{max}. One SD difference in ^{2}log(IGFBP-2) and ^{2}log(TIMP4) was associated with a mean difference of 1.04 mm/m^2 (95% CI 0.53–1.56) and 1.01 mm/m^2 (0.50–1.52) in ID_{max}, respectively.

Supplemental file 1 shows the results of univariable and multivariable analysis of the four biomarkers associated with the study endpoints AD_{max} or ID_{max}. Higher CHI3L1 and MMP-3 levels were significantly associated with AD_{max} in univariable analysis. Multivariable adjustment for age and sex blunted the association between CHI3L1 and AD_{max}. MMP-3 remained significantly associated with AD_{max}, even after additional adjustment for previous aortic dissection and previous aortic surgery. Higher IGFBP-2 and Tissue Inhibitor of Metalloproteinases 4 (TIMP4) levels were significantly associated with ID_{max} in univariable analysis. Multivariable adjustment for age and sex blunted the association between TIMP4 and ID_{max}, whereas IGFBP-2 remained significantly associated with ID_{max}. In supplemental file 2, a visualization is represented of the association between the four abovementioned biomarkers and the study endpoints. Stratified univariable analyses for males and females are shown in supplemental file 3, which shows IGFBP-2 is significantly associated with ID_{max} in females but not in males. Whereas the other previously mentioned biomarkers: TIMP4, MMP-3 and CHI3L1 were only significant in the total population, and seem equally associated with AD_{max} and ID_{max} in males and females in univariable analysis.

When analyzing the maximal ascending and descending thoracic aortic diameters separately, no biomarkers showed a significant association with maximal ascending aortic diameter. However, Fatty-acid binding protein 4 (FABP4) showed a significant association with absolute maximal descending aortic diameter, even after correction for age, sex, dissection and surgery (supplemental file 4).

Comparison of biomarkers in different subgroups of TAD patients is shown in Figs. 3a and 3b. All biomarkers with a *p*-value <0.05 are presented in these figures. After Bonferroni correction, N-terminal-pro hormone BNP (NT-proBNP) was significantly higher in TAD patients with previous surgery and/or dissection (3.67 [3.01–3.99] vs 2.84 [2.32–3.26], $p \leq 0.001$). The median time between previous surgery and study inclusion was 52 months (IQR 12–90). In patients with known hereditary TAD (Fig. 3b), Trem-like transcript protein 2 (TLT-2) was significantly higher than in patients without confirmed hereditary TAD (4.64 [4.45–4.84] vs 4.40 [4.17–4.64], $p = 0.00042$). Hereditary TAD remained associated with higher TLT-2 levels after correction for sex, age, previous surgery, previous dissection and absolute TAD diameter (β [95%CI]: 0.27 [0.06–0.48], $p = 0.013$). Tables corresponding with Figs. 3a and 3b are shown in supplemental file 5.

From all patients with a history of thoracic aortic dissection ($n = 16$)

Table 1
Baseline characteristics.[i]

	Total (n = 158)	Males (n = 99)	Females (n = 59)	P-value	Missing (%)
Age - y	61.0 (50.3–68.8)	62.0 (52.0–69.0)	57.0 (47.5–66.0)	0.257	0.0
Height - cm	179 ± 11	185 ± 9	170 ± 7	<0.001*	0.0
Weight - kg	86.1 ± 15.2	92.0 ± 13.2	76.3 ± 13.3	<0.001*	0.6
BSA	2.1 ± 0.2	2.2 ± 0.2	1.9 ± 0.2	<0.001*	0.6
Hypertension	91 (57.6)	60 (60.6)	31 (52.5)	0.419	0.0
Hyperlipidaemia	50 (31.6)	40 (40.4)	10 (16.9)	0.005*	0.0
Smoking	15 (12.9)	7 (9.6)	8 (18.6)	0.266	26.6
Diabetes	6 (3.8)	5 (5.1)	1 (1.7)	0.412[i]	0.0
Renal dysfunction	5 (3.2)	3 (3.0)	2 (3.4)	1.000[i]	0.0
LVEF[1]				0.359	0.0
Normal	91 (57.6)	62 (62.6)	29 (49.2)		
Slightly reduced	10 (6.3)	5 (5.1)	5 (8.5)		
Moderately reduced	1 (0.6)	1 (1.0)	0 (0.0)		
Severely reduced	1 (0.6)	1 (1.0)	0 (0.0)		
Medication					0.0
Beta blocker	55 (34.8)	40 (40.4)	15 (25.4)	0.082	
ACEi	34 (21.5)	26 (26.3)	8 (13.6)	0.093	
ARB	27 (17.1)	20 (20.2)	7 (11.9)	0.259	
Diuretics	27 (17.1)	17 (17.2)	10 (16.9)	1.000	
Cholesterol	46 (29.1)	35 (35.4)	11 (18.6)	0.040*	
Antithrombotics	63 (39.9)	48 (48.5)	15 (25.4)	0.007*	
Hereditary TAD diagnosis				0.160	0.0
Marfan syndrome	11 (7.0)	9 (9.1)	2 (3.4)		
Loeys-Dietz syndrome	18 (13.4)	7 (7.1)	11 (18.7)		
Ehlers-Danlos syndrome	3 (1.9)	1 (1.0)	2 (3.4)		
Other	4 (2.5)	3 (3.0)	1 (1.7)		
Bicuspid aortic valve	6 (3.8)	6 (6.1)	0 (0.0)		
Genetic mutation					
SMAD3	11 (7.0)	5 (5.1)	6 (10.2)		
VUS	9 (5.7)	6 (6.1)	3 (5.1)		
TGFB3	4 (2.5)	2 (2.0)	2 (3.4)		
TGFB2	1 (0.6)	0 (0.0)	1 (1.7)		
Other	5 (3.2)	3 (3.0)	2 (3.4)		
Abdominal aortic aneurysm	11 (7.0)	9 (9.1)	2 (3.4)	0.212	0.0
Other arterial aneurysm	18 (11.4)	9 (9.1)	9 (15.3)	0.357	0.0
AD_{max} (mm)	43.3 ± 5.4	44.0 ± 5.4	42.0 ± 5.1	0.021*	3.8
ID_{max} (mm/m^2)	21.3 ± 3.3	20.6 ± 3.0	22.5 ± 3.5	0.001*	4.4
Previous aortic surgery	30 (19.0)	20 (20.2)	10 (16.9)	0.768	
Previous dissection	16 (10.1)	8 (8.1)	8 (13.6)	0.412	0.6

Data are expressed as mean ± SD or as absolute and percentage. BSA = Body Surface Area; ARB = Angiotensin II receptor blocker; ACEi = Angiotensin Converting Enzyme inhibitor; LVEF = left ventricular ejection fraction; AD_{max} = Maximal absolute diameter thoracic aorta; ID_{max} = Maximal indexed diameter thoracic aorta.
[i] Fishers Exact test.
[1] Only available in patients who received echocardiography at baseline visit n = 104.
* Significant at the 0.05 level.

four patients (2.5%) had Stanford type A aortic dissections, and 12 patients (7.5%) had type B aortic dissections. In this subgroup of patients with thoracic aortic dissection, no biomarkers were significantly associated with either AD_{max} or ID_{max} after Bonferroni correction. However, there was a trend towards a significant association between AD_{max} and FABP4 (p = 0.00418) and between ID_{max} and FABP4 (p = 0.00106). In the subgroup analyses for HTAD patients, no biomarkers were significantly associated with AD_{max} or ID_{max} after Bonferroni correction.

4. Discussion

In this study, we evaluated the potential association of 91 biomarkers related to cardiovascular disease, with thoracic aortic diameter in TAD patients. Although several proteins involved in proteolysis and inflammation were found to be associated with aortic diameter, it is important to keep in mind that this kind of analysis is merely hypothesis generating. More research is needed to establish the actual relation between these biomarkers and TAD severity. MMP-3 was found to be associated with AD_{max}. IGFBP-2 was significantly associated with ID_{max} in females. Furthermore, TLT-2 was found to be significantly higher in patients with hereditary TAD.

This study shows a positive association between AD_{max} and plasma levels of MMP-3. Matrix metalloproteinases (MMP's) regulate the degradation of elastin and collagen. Plasma levels of several MMP's have been reported to be elevated in TAD patients [13]. Additionally, some MMP's in the thoracic aortic wall were found to be higher in patients with TAD and aortic dissection than in controls [14]. MMP-3 has not previously been reported as elevated or decreased in TAD patients or in association to the thoracic aortic diameter [13]. However, an inverse association between MMP-3 expression in the aortic wall and elasticity of the TAD wall has been reported [15]. Reduced aortic wall elasticity could potentially promote aneurysm formation. Therefore it is not surprising that we found circulating MMP-3 levels to be positively associated with thoracic aortic diameters. The tissue inhibitors of MMP's are TIMP's. We found a positive association between ID_{max} and TIMP-4 in univariable analysis, which was not significant anymore after adjustment for sex and age. Previous studies using gene expression profiling show decreased rather than elevated expression of TIMP4 in aortic specimens of patients with aortic dissection, which seems logical since this would lead to higher MMP levels and more degradation of elastin and collagen weakening the aortic wall. Therefore we are indeed not convinced of a true association between TIMP4 and aortic dilatation [16].

CHI3L1 was positively associated with AD_{max} in univariable analysis. CHI3L1 seems to plays a major role in tissue injury, inflammation, tissue repair, and remodeling responses [17]. CHI3L1 has been associated with various diseases among which AAA, coronary- and carotid atherosclerosis, cancer and several neurological disorders [17,18]. So far, no

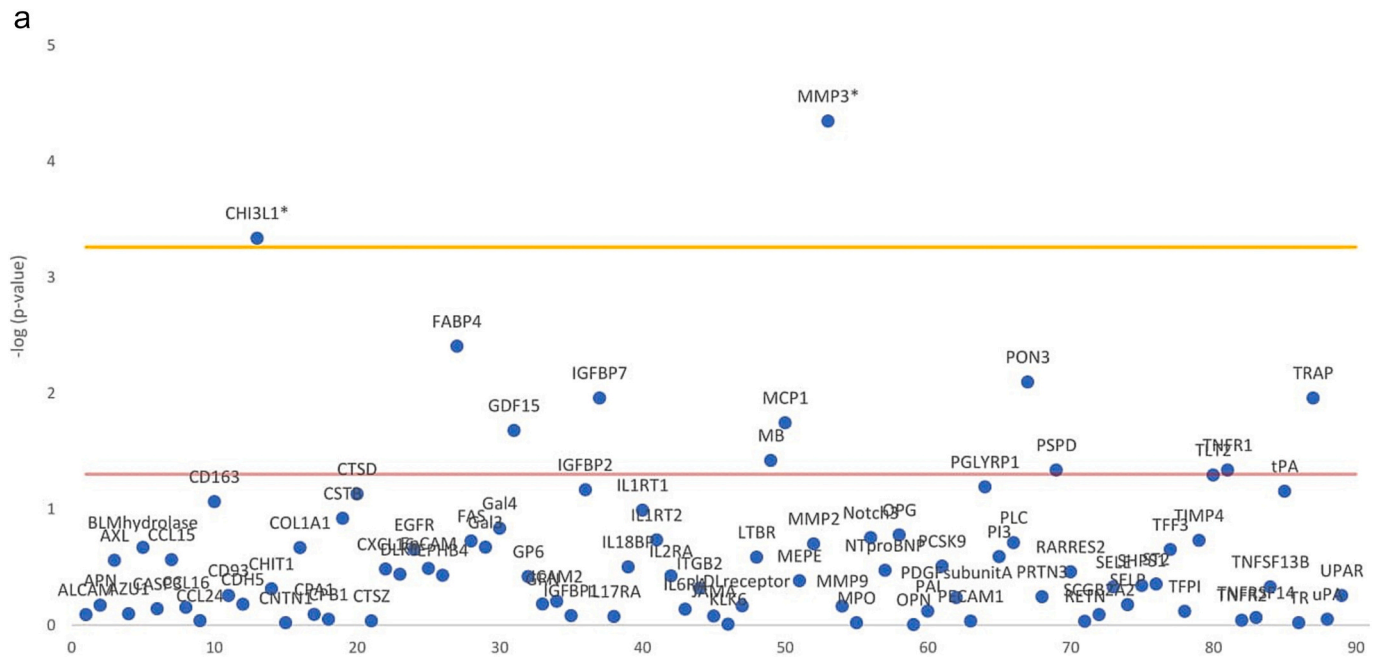

Fig. 2a. Univariable analysis of 91 biomarkers with absolute thoracic aortic diameter.

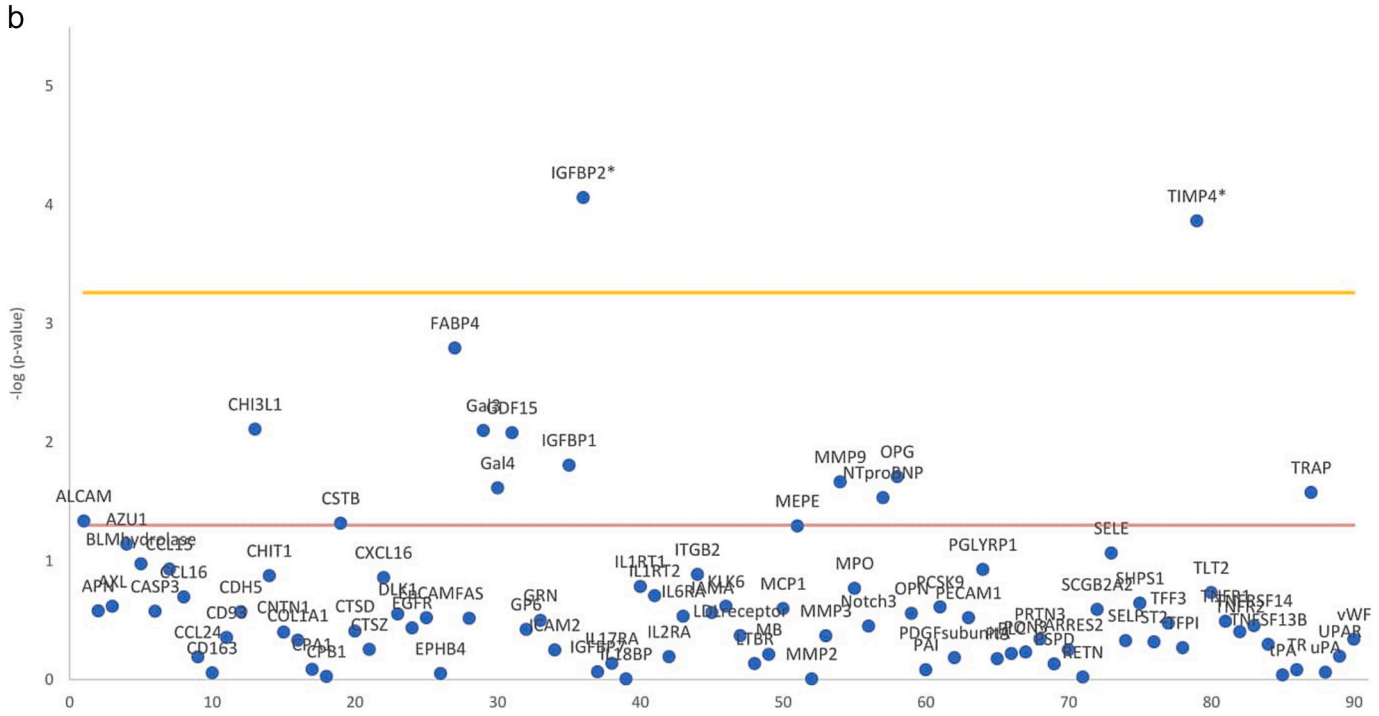

Fig. 2b. Univariable analysis of 91 biomarkers with indexed thoracic aortic diameter.
The yellow line represents the Bonferroni corrected *p*-value. Biomarkers above the yellow line showed a statistically significant association with AD_{max} (Fig. 1a) or ID_{max} (Fig. 1b) in univariable linear regression analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

studies investigated plasma measurements of CHI3L1 in TAD patients. Our study showed no significant association between AD_{max} or ID_{max} after adjustment for sex and age. This suggests CHI3L1 is not a potential plasma biomarker for diagnosis of TAD.

IGFBP2 showed a significant positive association with ID_{max} in females with TAD. IGFBP-2 is a protein that regulates transport and bioavailability of Insulin-like Growth Factor 1 (IGF-1). In the heart, IGF-1 regulates several cellular processes including metabolism, apoptosis, ageing, and growth [19]. In the circulation, IGFBP-2 predominately has an inhibitory effect on IGF-1. In cardiovascular research IGFBP-2 is regarded a rather novel biomarker, which has shown potential for diagnosis and prognosis of heart failure, and for use of risk prediction in patients needing transcatheter aortic valve implantation [20,21]. Circulating IGFBP-2 has not previously been reported in association to TAD. Our finding suggests IGFBP-2 might also have an association with TAD severity, especially in females with TAD, warranting further male-

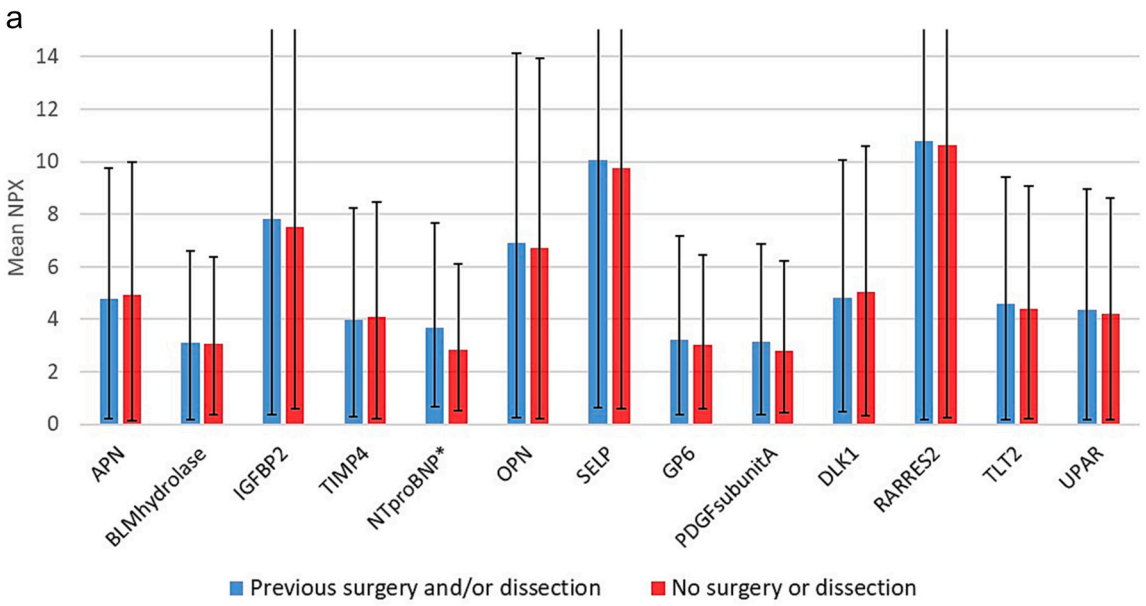

Fig. 3a. Comparison of biomarkers between TAD patients with and without previous surgery and/or dissection.
TAD = Thoracic Aortic Disease; APN = aminopeptidase; NPX = Normalized Protein Expression units; IGFBP2 = Insulin-like growth factor binding protein 2; TIMP4 = Metalloproteinase inhibitor 4; NTproBNP = N-terminal prohormone brain natriuretic peptide; OPN = Osteopontin; SELP = P-selectin; GP6 = Pletelet glycoprotein VI; PDGF subunit A = Platelet-derived growth factor subunit A; DLK1 = Protein delta homolog 1; RARRES2 = Retinoic acid receptor responder protein 2; TLT2 = Trem-like transcript 2 protein; UPAR = Urokinase plasminogen activator surface receptor.
* = Significant after Bonferroni correction.

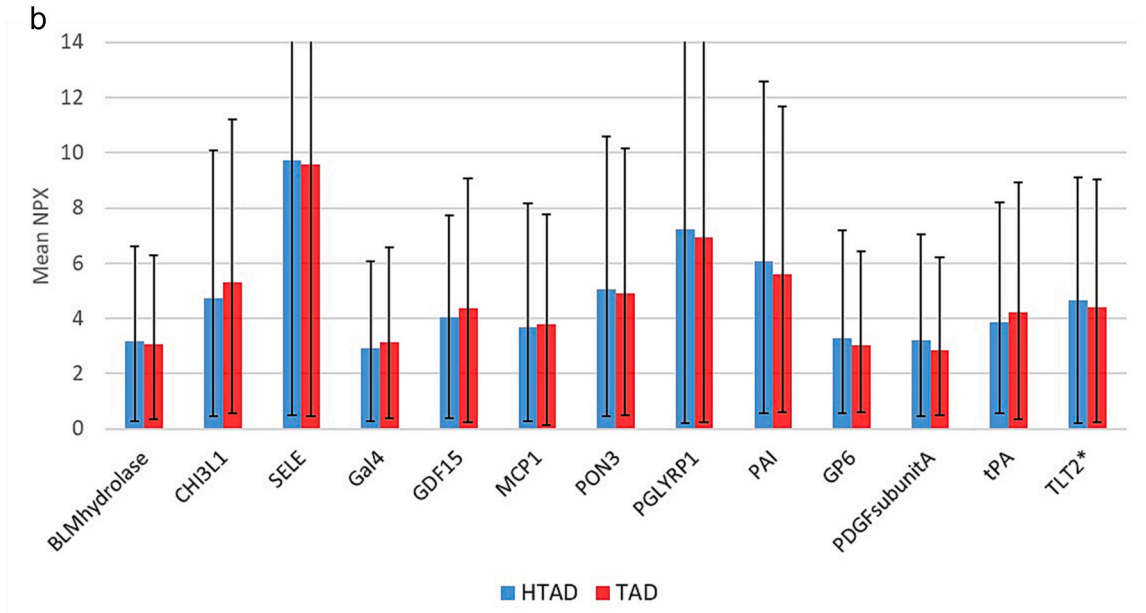

Fig. 3b. Comparison of biomarkers between Hereditary TAD patients and non-hereditary TAD patients.
TAD = Thoracic Aortic Disease; NPX = Normalized Protein Expression units; CHI3L1 = Chitinase-3-like protein 1; SELE = *E*-selectin; Gal4 = Galectin-4; GDF15 = Growth/differentiation factor 15; MCP1 = Monocyte chemotactic protein 1; PON3 = Paraoxonase; PGLYRP1 = Peptidoglycan recognition protein 1; PAI = Plasminogen activator inhibitor 1; GP6 = Pletelet glycoprotein VI; PDGF subunit A = Platelet-derived growth factor subunit A; tPA = Tissue-type plasminogen activator; TLT2 = Trem-like transcript 2 protein.
* = Significant after Bonferroni correction.

female specific research.

We found plasma TLT-2 was associated with the presence of hereditary TAD, even after correction for thoracic aortic diameter. TLT-2 is expressed in cells of the immune system, such as T-cells and B-cells, neutrophils and macrophages [22]. TLT-2 is involved in leukocyte activation, and expression of TLT-2 is up-regulated in response to inflammatory stimuli [23]. Evidence suggests inflammatory responses are involved in TAD pathogenesis, and therefore it has been claimed that TAD should be seen as an inflammatory disease [24]. Moreover, among patients with Loeys-Dietz Syndrome (LDS), a rare hereditary TAD, there is a high prevalence of immunologic features including osteoarthritis, asthma, food allergy, eczema and allergic rhinitis [25]. Also in Marfan

Syndrome contribution of inflammation in the development of aortic dilatation and many of the other clinical features has been reported [26]. Several aspects of the Triggering Receptor Expressed on Myeloid cells (TREM) cascade, which includes TLT-2, have been linked to these clinical features [27]. Elevated TLT-2 levels in hereditary TAD patients might be another indication of inflammatory involvement in thoracic aortic disease and connective tissue disorders. However, it remains unclear why TLT-2 is the only inflammatory biomarker which was significantly higher in hereditary TAD patients. Indeed also other factors involved in inflammation were expected to be higher. Such as monocyte chemotactic protein 1 (MCP-1) which has been found to be associated with cerebral and abdominal aneurysm formation and aortic dissection in mice [28]. This might be explained by the fact that we included a small and heterogeneous sample of hereditary TAD patients, or TLT-2 could be a more specific biomarker for hereditary TAD patients. The exact role of TLT-2 in the processes of different connective tissue diseases causing hereditary TAD needs further attention.

FABP4, also known as adipocyte FABP (A-FABP), is a fatty-acid binding protein which regulates lipid trafficking and responses in cells. FABP4 is highly expressed in adipocytes, but also in macrophages and dendritic cells. FABP4 has been associated with various cardiovascular diseases, including diabetes mellitus, hypertension [29], adiposity and atherosclerosis [30]. In a recent study by Memon et al., which used the same Olink panel for analyzing biomarkers, FABP4 was found to correlate with absolute abdominal aortic aneurysm diameter [9]. In our cohort FABP4 was associated with absolute descending aortic diameter and showed a trend towards significance in a subgroup of patients with thoracic aortic dissection. It seems FABP4 could be a potential prognostic marker for descending thoracic aortic pathology. Although elevated FABP4 levels do not seem very specific for aortic pathology, agents capable of modifying FABP4 function could become a new class of therapeutic agents against several metabolic and cardiovascular diseases. Since these agents have already been developed, and have shown beneficial for preventing atherosclerosis and outcome of ischemic stroke in mouse model studies [31,32].

This is the first study that used a relatively new technology, the proximity extension assay, to evaluate a large amount of biomarkers associated with cardiovascular diseases and their association with TAD. We identified several promising biomarkers which originate from proteolysis and inflammation pathways. Further studies should evaluate the potential clinical use of these biomarkers for diagnosis and surveillance of TAD. Moreover, these biomarkers can help reveal biochemical pathways in pathogenesis of TAD, which could provide options for pharmaceutical therapies.

4.1. Limitations

Our study has several limitations. First, this study was performed in TAD patients only, which means we were unable to compare biomarkers between TAD patients and a population without TAD. Therefore we assessed only the biomarkers associated with thoracic aortic diameter as an indication of TAD severity. A sample size calculation was performed using nQuery [33], which showed a sample size of 400 is necessary to reproduce most of our results. However, we believe it might be more valuable to compare biomarker levels in TAD patients to control population in future studies. In this case, a smaller sample size will be sufficient. Second, we used biomarker values in Normalized Protein Expression (NPX) Units, i.e., relative units. While these values can be used for comparing patients and changes over time within a patient, for clinical applications absolute concentrations are recommended. Third, CT imaging was not available in 52 patients (32.9%), in these patients aortic diameter was measured using TTE, which might be slightly less accurate. However, this variability in imaging modalities allowed us to perform blood sampling and imaging on the same day. Last, our tertiary care facility is a referral center for patients with hereditary thoracic aortic disease. This might have resulted in a relatively large amount of patients with HTAD in our cohort, which could have influenced our results. As information on biomarkers is extremely limited in patients with aortic disease, it is not known whether biomarkers differ between HTAD patients and non-syndromic TAD patients.

5. Conclusions

We identified MMP-3, IGFBP-2 and FABP4 as plasma biomarkers associated with thoracic aortic diameter in TAD patients. Elevated TLT-2 levels might indicate inflammatory involvement in hereditary TAD patients. These biomarkers and their corresponding biochemical pathways seem to play a role in assessing TAD severity. The potential clinical use of these biomarkers and their biochemical pathways warrants further research.

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Authors' contributions

Carlijn Thijssen provided substantial contributions to the conception, design, data collection, data analysis, interpretation of the data and preparation of the manuscript. Silvy Dekker provided substantial contributions to the design and data collection of this study. Jolien Roos-Hesselink supervised this project from its conception, including the study design, data analysis and interpretation, and manuscript preparation. These authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Roland van Kimmenade and Eric Boersma co-supervised the projects data analysis and interpretation, and manuscript preparation. Lidia Bons, Laurie Geenen, Arjen Gökalp, Johanna Takkenberg, Mostafa Mokhles, Jos Bekkers and Elke Bouwens provided substantial contributions to the conception, design, and drafting of this work, aided in revising the work critically for important intellectual content, and provided final approval of the version to be published. All authors consented to the submission of this manuscript.

Declaration of Competing Interest

Authors Carlijn Thijssen, Silvy Dekker, Lidia Bons, Laurie Geenen, Arjen Gökalp, Johanna Takkenberg, Mostafa Mokhles, Jos Bekkers, Elke Bouwens, Eric Boersma, Roland van Kimmenade and Jolien Roos-Hesselink declare that they have no conflict of interest.

Data availability

Data will be shared on request to the corresponding author.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2023.02.006.

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