Circulating fibrillin fragment concentrations in patients with and without aortic pathology

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ABSTRACT

Objective: Fragments of fibrillin-1 and fibrillin-2 will be detectable in the plasma of patients with aortic dissections and aneurysms. We sought to determine whether the plasma fibrillin fragment levels (PFFLs) differ between patients with thoracic aortic pathology and those presenting with nonaortic chest pain.

Methods: PFFLs were measured in patients with thoracic aortic aneurysm (n = 27) or dissection (n = 28). For comparison, patients without aortic pathology who had presented to the emergency department with acute chest pain (n = 281) were categorized into three groups according to the cause of the chest pain: ischemic cardiac chest pain; nonischemic cardiac chest pain; and noncardiac chest pain. The PFFLs were measured using a sandwich enzyme-linked immunosorbent assay.

Results: Fibrillin-1 fragments were detectable in all patients and were lowest in the ischemic cardiac chest pain group. Age, sex, and the presence of hypertension were associated with differences in fibrillin-1 fragment levels. Fibrillin-2 fragments were detected more often in the thoracic aneurysm and dissection groups than in the emergency department chest pain group ($P < .0001$). Patients with aortic dissection demonstrated a trend toward increased detectability ($P = .051$) and concentrations ($P = .06$) of fibrillin-2 fragments compared with patients with aortic aneurysms. Analysis of specific antibody pairs identified fibrillin-1 B15-HRP26 and fibrillin-2 B205-HRP143 as the most informative in distinguishing between the emergency department and aortic pathology groups.

Conclusions: Patients with thoracic aortic dissections demonstrated elevated plasma fibrillin-2 fragment levels (B205-HRP143) compared with patients presenting with ischemic or nonischemic cardiac chest pain and increased fibrillin-1 levels (B15-HRP26) compared with patients with ischemic cardiac chest pain. Investigation of fibrillin-1 and fibrillin-2 fragment generation might lead to diagnostic, therapeutic, and prognostic advances for patients with thoracic aortic dissection. (JVS—Vascular Science 2022;3:389-402.)

Clinical Relevance: Differentiating between thoracic aortic dissection and other causes of acute chest pain is clinically important. Investigating the clinical and mechanistic processes that contribute to higher fibrillin fragment concentrations in patients with thoracic aortic dissection compared with patients with cardiac chest pain could lead to diagnostic, prognostic, and therapeutic improvements in the care of patients with thoracic aortic dissections.

Keywords: Aortic dissection; Biomarkers; Chest pain; Fibrillin

Patients with acute aortic dissection and other acute thoracic aortic pathology often present with chest pain. At present, no reliable biomarkers are available that can detect or exclude aortic pathology in patients presenting with chest pain. Therefore, cross-sectional imaging with intravenous contrast will generally be required. Fibrillin-

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The present study was supported by the National Heart, Lung, and Blood Institute (grant 1R43HL147757 to J.S.K.).

Author conflict of interest: L.Y.S. and E.J.C. are co-inventors and hold a patent titled "Profiling fragments of elastic fibers and microfibrils as biomarkers for disease" (Sakai et al, 2018; U.S. patent no. 9,857380); Virogenomics BioDevelopment, Inc, holds an exclusive license to develop the technology described in this patent. M.E., D.D., T.C., R.L.S., J.S.K., K.N., G.J.L., G.L.M., C.A., and A.A. have no conflicts of interest.

Accepted for presentation at the 2020 Vascular Annual Meeting of the Society for Vascular Surgery, Toronto, ON, Canada, June 17-20, 2020 (conference cancelled). Presented as an interactive poster session at the 2020 Society for Vascular Surgery, ONLINE, June 17-July 20, 2020.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS-Vascular Science policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2666-3503

https://doi.org/10.1016/j.jvssci.2022.09.001

1 and fibrillin-2 are extracellular matrix (ECM) structural components of muscular arteries, and plasma fibrillin-1 and fibrillin-2 fragment levels (PFFLs), presumably derived from the breakdown of tissue fibrillin-1 and fibrillin-2, will be elevated in patients with thoracic aortic aneurysms (TAAs) and thoracic aortic dissections (TADs) compared with control patients without aortic pathology.[1,2] Moreover, pathogenic variants in *FBN1*, the gene for fibrillin-1, cause Marfan syndrome.[3] Individuals with Marfan syndrome are disposed to the development of both aortic aneurysms and dissections and will demonstrate progressive degradation of aortic fibrillin with aneurysm formation.[4] Because PFFLs appear to be higher in patients with aortic aneurysms and dissections, interest has ensued in the use of PFFLs as a biomarker for acute aortic pathology.[2,5] However, it is unknown whether other cardiovascular conditions that result in chest pain, such as myocardial infarction and angina, will affect PFFLs. The utility of PFFLs as a potential biomarker for acute aortic pathology will depend on the ability to distinguish between patients with acute aortic pathology and those presenting with other causes of acute chest pain.

Although previous studies have demonstrated that circulating fibrillin-1 fragments can be associated with thoracic aortic dissection, fibrillin-1 and fibrillin-2 are present in many tissues throughout the body; thus, fibrillin fragments can be derived from both arterial and nonarterial sources.[1,2] The exact mechanisms of fibrillin-1 and fibrillin-2 degradation in the aorta and other tissues are not understood, and the structure of the resulting fragments is not known. Different degradative processes will likely produce different circulating fibrillin fragments, which might not contain all the domains of an intact fibrillin monomer, and PFFLs could depend on the specific epitopes targeted by the detector antibody pairs. Therefore, we sought to determine whether antibodies to different fibrillin epitopes can distinguish between PFFLs in patients with thoracic aortic pathology and those presenting with nonaortic chest pain using antibody pairs to six different epitopes of fibrillin-1 and fibrillin-2.

ARTICLE HIGHLIGHTS

- **Type of Research:** A single-center, prospective, case-control study
- **Key Findings:** A comparison of circulating fibrillin fragment levels between patients with thoracic aortic dissection (n = 28), thoracic aortic aneurysms (n = 27), and nonaortic chest pain (n = 281) demonstrated significantly higher fragment fibrillin fragment levels in the patients with thoracic aortic dissection than in the patients presenting with cardiac chest pain.
- **Take Home Message:** Patients with thoracic aortic dissection demonstrated higher circulating fibrillin fragment concentrations than had patients with chest pain of cardiac origin.

METHODS

Our institutional review board approved the present study, and all participants provided written informed consent.

Study participants

Emergency department group. All patients aged >18 years and without a known pregnancy who had presented to our tertiary care emergency department (ED) with a chief complaint of chest pain were approached for participation in the present study. A total of 281 patients were enrolled in the ED group. After enrollment, those patients with known aortic aneurysms, cancer, or an inadequate blood sample were enrolled but were excluded from the final analysis to exclude confounding factors that could affect fibrillin levels in patients presenting with acute chest pain. Of the seven patients with a known aortic aneurysm and excluded from the final analysis, two had had a diagnosis of ischemic cardiac chest pain, one a diagnosis of nonischemic cardiac chest pain, and three, noncardiac chest pain. The final patient had had a symptomatic ascending aortic aneurysm. All the patients had had whole blood samples drawn into heparin tubes, processed immediately into plasma, and stored at −80°C.

The demographic data, medical comorbidities, and determined cause of chest pain were obtained from the electronic medical records. These patients were categorized into three groups according to the cause of chest pain: group 1, ischemic cardiac chest pain (ICCP); group 2, non-ICCP (NICCP); and group 3, noncardiac chest pain (NCCP). The ICCP group included patients who had presented with myocardial infarction or angina. The diagnosis of ICCP was determined by the serum troponin level, electrocardiographic changes, and final diagnosis at ED visit. All patients in the ICCP group had had troponin levels available for review. The NICCP group included patients who had presented with arrhythmia, heart failure, hypertensive urgency, and pericarditis, myocarditis, or endocarditis. The NCCP group included all patients with chest pain not attributable to the cardiovascular system or aorta.

TAA and TAD group. Patients with a TAA or TAD who had presented to either the ED or vascular surgery clinic were approached for participation in the present study. A total of 55 patients (27 with a TAA and 28 with a TAD) were enrolled. Within the TAD group, 6 patients had had aneurysmal degeneration of the dissected aorta and 22 had had aortic dissection without aneurysmal degeneration. The TAD group was further subclassified

as having an acute (≤14 days), subacute (15 days to 6 months), or chronic (>6 months) aortic dissection. Within the TAD group, 6 patients had had acute and subacute aortic dissection and 22 had had chronic aortic dissection. The TAD group included 15 type B dissections and 13 type A dissections. The TAD group did not contain any patients with penetrating aortic ulcers. The TAA group included 11 patients with ascending aortic aneurysms and 16 with descending aortic aneurysms.

Fibrillin assay

The PFFLs were determined using a modified sandwich enzyme-linked immunosorbent assay with biotinylated capture antibodies and horseradish peroxidase (HRP)-conjugated detector antibodies. The epitopes for the antibodies used in the present study were mapped to the fibrillin domains shown in the Supplementary Fig. We coated 96-well Costar plates (model no. 3950; Corning, Corning, NY) with 100 μL/well of either 5 μg/mL or 10 μg/mL streptavidin (Pierce; Thermo Fisher Scientific, Waltham, MA) in carbonate/bicarbonate coating buffer overnight at 4°C with agitation. Unbound material was removed by washing three times with 300 μL of Tris-buffered saline, 0.025% Tween-20 (TBST) using a Biotek EL×50 plate washer. Next, 100 μL/well of biotinylated monoclonal capture antibody (concentration 0.25 μg/mL for B15 and 1 μg/mL for B201, B205, and B72) was added to each plate, and the plates were incubated for 1 hour at 25°C with agitation. After washing to remove unbound antibody, the standards and samples were added in duplicate and triplicate, respectively. The plates were loaded with 10 μL of heparinized plasma plus 90 μL of TBST. The plates for the antibody pairs B15-HRP78 received 4 μL of heparinized plasma plus 96 μL of TBST. Standard curves were developed using recombinant fibrillin-1 peptide rF11 or recombinant fibrillin-2 peptide rF37 titrated over a range of 0.5 to 0 μg/mL in 12 wells. The plates were incubated overnight at 4°C with agitation. After washing, 100 μL/well of HRP-conjugated monoclonal detector antibody was added to each plate (concentration 0.05 μg/mL for HRP201, 0.5 μg/mL for HRP26, 0.3 μg/mL for HRP78, 1 μg/mL for HRP78, 1 μg/mL for HRP143, and 0.1 μg/mL for HRP143), and the plates were incubated for 1 hour at 25°C with agitation. After washing, 50 μL of TMB (3,3′,5,5′-tetramethylbenzidine) substrate (equilibrated to room temperature) was added to each well. After incubating for 15 minutes at room temperature, 50 μL of 2N sulfuric acid was added to each well to stop the reaction. The plates were read at 450 nm using a Spectramax i3x plate reader (Molecular Devices, San Jose, CA).

The concentrations of PFFLs were calculated from the average absorbance of each sample in triplicate and converted to μg/mL with an equation generated from the standard curves. The lower limit of detection was defined as the value two standard deviations above the mean absorbance of blank wells. The results from each of four antibody pairs specific for fibrillin-1 epitopes (B15-HRP201, B15-HRP26, B15-HRP78, and B201-HRP78) were assessed according to the standard curves using recombinant fibrillin-1 polypeptide rF11. The results from two antibody pairs specific for fibrillin-2 epitopes (B205-HRP143 and B72-HRP143) were assessed according to the standard curves using recombinant fibrillin-2 polypeptide rF37. Detailed methods for fitting concentrations to standard curves were described by Marshall et al.[2] Each enzyme-linked immunosorbent assay plate contained the appropriate standard curve for the capture-detector antibody pair used for testing the triplicate samples on the plate. A four-parameter log logistic curve was fit to the standards, and a goodness-of-curve fit was determined. Standards were required to be within ±15% of the calculated recovery value, or the samples were retested.

In addition to calculating concentrations using individual pairs of antibodies, the maximal fragment concentration for fibrillin-1 and the maximal fibrillin-2 concentration, coded as the highest concentration observed among the four fibrillin-1 antibody pairs and the highest concentration among the two fibrillin-2 pairs, respectively, was compared between the groups. The maximal fragment concentration was used, rather than averaging the concentration of the four fibrillin-1 fragments and the two fibrillin-2 fragments to avoid diluting the results of the fragments with significant intergroup differences with the results of fragments without significant intergroup differences.

Statistical analysis

Statistical analysis was performed using Stata IC, version 13.1 (StataCorp, College Station, TX). The categorical variables were compared using the Fisher exact test. The fibrillin fragment concentrations across the groups were compared using either the Mann-Whitney *U* test or Kruskal-Wallis test. The Dunn post hoc test (with Bonferroni adjustment) was used to determine whether the pairwise comparisons were significant after the Kruskal-Wallis test. Spearman's correlation was used to test for a monotonic association between untransformed continuous variables. A P value of $< .05$ was used as a cutoff for statistical significance. Graphs were created using Prism, version 5.02 (GraphPad Software, San Diego, CA).

RESULTS

Demographics and medical histories of ED patients.

The 281 ED patient samples were assessed for the PFFLs. Of the 281 patients, 18 were excluded from the analysis because of the presence of an aortic aneurysm (n = 7), cancer (n = 7), pregnancy (n = 1), or recent surgery (n = 3). The cause of chest pain was determined as ICCP for 30, NICCP for 39, and NCCP for 194 patients. The demographic variables and comorbidities within each diagnosis group

Table I. Distribution of demographic factors and medical history stratified by diagnosis group

Variable	ICCP (n = 30)	NICCP (n = 39)	NCCP (n = 194)	*P* value[a]
Age, years	63 ± 9.7	54 ± 17.5	50 ± 17.3	.0003
Gender				.037
Male	21 (70)	23 (59)	91 (47)	
Female	9 (30)	16 (41)	103 (53)	
BMI, kg/m^2	29.2 ± 5.2	30.7 ± 8.1	30.9 ± 9.8	.91
Medical history				
Hyperlipidemia	18 (60)	17 (44)	66 (34)	.020
Hypertension	23 (77)	22 (56)	98 (51)	.024
Diabetes mellitus	12 (40)	11 (28)	35 (18)	.020
COPD	2 (7)	4 (6)	12 (6)	.56
Peripheral artery disease	2 (7)	0 (0)	5 (3)	.19
Stroke	2 (7)	6 (15)	19 (10)	.50
Vascular procedures	4 (13)	1 (3)	12 (6)	.22
ESRD	0 (0)	2 (5)	5 (3)	.43
Dialysis	0 (0)	2 (5)	5 (3)	.54
CAD	14 (47)	8 (21)	30 (15)	.001
Angina	20 (67)	3 (8)	12 (6)	<.0001
MI	12 (40)	5 (13)	24 (12)	.002
Arrhythmia	3 (10)	12 (31)	53 (27)	.085
CHF	4 (13)	10 (26)	23 (12)	.089
Cardiac procedures	15 (50)	10 (26)	35 (18)	.001

BMI, Body mass index; *CAD,* coronary artery disease; *CHF,* congestive heart failure; *COPD,* chronic obstructive pulmonary disease; *ESRD,* end-stage renal disease; *ICCP,* ischemic cardiac chest pain; *MI,* myocardial infarction; *NCCP,* noncardiac chest pain; *NICCP,* nonischemic cardiac chest pain. Data presented as mean ± standard deviation or number (%).
[a]Fisher's exact test used to assess *P* values, except for age and BMI, which were analyzed using the Kruskal-Wallis test for continuous variables.

are listed in Table I. The ICCP patients were older, more likely to be men, and more likely to have had history of coronary artery disease (CAD) and several CAD-associated comorbidities (Table I).

Detectability and maximum fibrillin concentrations in ED study groups. All 263 patient plasma samples had had detectable levels of fibrillin-1 fragments, and 174 patient samples (66%) had had detectable fibrillin-2 fragment levels. The numbers of patients with detectable fragments of fibrillin-1 and fibrillin-2 are presented in Table II. The detectability of any fibrillin-2 fragment and fibrillin fragments B15-HRP78 and B205-HRP143 differed significantly between patient groups (Table II).

The concentrations of fibrillin fragments were determined by fitting the average absorbance of the test samples to the standard curves, as previously described.[2] The concentrations were calculated for fragments defined by the individual capture-detector antibody pairs (see Table III). As described in the Methods section, the maximal concentrations for fibrillin-1 and fibrillin-2 were used to generate the results shown in Figs 1 and 2.

The mean and interquartile range for the concentrations of fibrillin-1 and fibrillin-2 in the three chest pain groups are shown in Fig 1, *A*. The patients in the ICCP group demonstrated lower median fibrillin-1 and fibrillin-2 fragment levels compared with the patients in the NICCP and NCCP groups. The patients in the NICCP group had had lower median fibrillin-2 fragment levels compared with patients in the NCCP group. Scatter plots of the data summarized in Fig 1, *A,* are shown in Fig 1, *B*. The results were significantly different between diagnosis groups (Kruskal-Wallis test; $P = .028$ for fibrillin-1 and $P = .004$ for fibrillin-2 concentrations). The Dunn post hoc test was used to determine whether the pairwise comparisons were significant, following the Kruskal-Wallis assessment. Post hoc testing showed that significantly lower concentrations were found in the ICCP group compared with the NCCP group for both fibrillin-1 and fibrillin-2. Significantly lower concentrations of fibrillin-2 fragments were also present in the NICCP group compared with the NCCP group.

Association of PFFLs with demographics and medical histories in ED groups. Significant associations were found between the chest pain groups and age, sex, and certain medical histories (Table I). Therefore, the association of PFFLs with these categories was investigated. The scatter plots of the distributions of fibrillin-1 and fibrillin-2 PFFLs stratified by hypertension (HTN), sex, and a history of diabetes are shown in Fig 2.

Table II. Detectability of plasma fibrillin fragments stratified by diagnosis group

Variable	ICCP (n = 30)	NICCP (n = 39)	NCCP (n = 194)	*P* value[a]
Any fibrillin-1	100 (30)	100 (30)	100 (194)	1.0
B15-HRP26	100 (30)	100 (39)	99 (192)	1.0
B15-HRP78	83 (25)	95 (37)	96 (187)	**.024**
B15-HRP201	100 (30)	100 (39)	99 (192)	1.0
B201-HRP78	80 (24)	87 (34)	93 (180)	.063
Any fibrillin-2	60 (18)	46 (18)	71 (138)	**.010**
B205-HRP143	53 (16)	46 (18)	68 (132)	**.017**
B72-HRP143	23 (7)	13 (5)	23 (44)	.38

G, Group; *ICCP*, ischemic cardiac chest pain; *NCCP*, noncardiac chest pain; *NICCP*, nonischemic cardiac chest pain; *TAA*, thoracic aortic aneurysm; *TAD*, thoracic aortic dissection.
Data presented as median (interquartile range; range).
Boldface *P* values represent statistical significance ($P < .05$).
ICCP, Ischemic cardiac chest pain; *NCCP*, noncardiac chest pain; *NICCP*, nonischemic cardiac chest pain.
Data presented as percentage (number).
Boldface *P* values represent statistical significance.
[a]Fisher's exact test was used to identify significant differences.

Table III. Plasma fibrillin-1 and fibrillin-2 concentrations stratified by diagnosis group

Fibrillin fragment	G1 (ICCP; n = 30)	G2 (NICCP; n = 39)	G3 (NCCP; n = 194)	G4 (TAA; n = 26[a])	G5 (TAD; n = 28)	*P* value[b]	Dunn post hoc test[c]
B15-HRP201	0.07 (0.06-0.09; 0.033-0.13)	0.08 (0.05-0.13; 0.036-0.32)	0.08 (0.06-0.11; 0.040-0.59)	0.09 (0.06-0.14; 0.05-0.24)	0.09 (0.07-0.14; 0.04-0.22)	.09	—
B15-HRP26	0.08 (0.06-0.09; 0.039-0.19)	0.08 (0.06-0.13; 0.040-0.95)	0.09 (0.07-0.12; 0.037-0.60)	0.10 (0.07-0.14; 0.06-0.38)	0.11 (0.07-0.13; 0.05-0.5)	**.02**	G1 vs G3, $P = .03$; G1 vs G5; $P = .02$
B15-HRP78	0.09 (0.07-0.14; 0.039-0.58)	0.12 (0.09-0.21; 0.022-5.73)	0.13 (0.09-0.24; 0.039-29.2)	0.12 (0.09-0.22; 0-19.7)	0.13 (0.10-0.21; 0-0.94)	**.03**	G1 vs G3, $P = .007$
B201-HRP78	0.08 (0.04-0.68; 0-3.04)	0.14 (0.06-0.61; 0-20.2)	0.21 (0.08-0.69; 0-62.7)	0.11 (0.05-0.33; 0-45.7)	0.24 (0.06-0.63; 0-2.93)	.09	—
B205-HRP143	0.12 (0.05-0.15; 0-1.52)	0.1 (0.04-0.16; 0-54.7)	0.17 (0.08-0.37; 0-80.8)	0.21 (0.03-0.34; 0-35.4)	0.30 (0.13-0.49; 0-7.59)	**.001**	G1 vs G5, $P = .004$; G2 vs G5, $P = .002$
B72-HRP143	0.01 (0.007-0.02; 0-0.036)	0.008 (0.006-0.01; 0-0.14)	0.01 (0.006-0.02; 0-0.22)	0 (0-0.03; 0-0.20)	0.006 (0-0.03; 0-0.05)	.25	—

G, Group; *ICCP*, ischemic cardiac chest pain; *NCCP*, noncardiac chest pain; *NICCP*, nonischemic cardiac chest pain; *TAA*, thoracic aortic aneurysm; *TAD*, thoracic aortic dissection.
Data presented as median (interquartile range; range).
Boldface *P* values represent statistical significance ($P < .05$).
ICCP, Ischemic cardiac chest pain; *NCCP*, noncardiac chest pain; *NICCP*, nonischemic cardiac chest pain.
Data presented as percentage (number).
Boldface *P* values represent statistical significance.
[a]For G4, n = 27 for B201-HRP78.
[b]Kruskal-Wallis test.
[c]Dunn post hoc test revealed statistically significant differences between specific groups.

Significantly lower fibrillin-1 concentrations were detected between the groups when stratified by HTN and sex (Kruskal-Wallis test; $P = .029$ and $P = .011$, respectively) but not for a history of diabetes. The post hoc test found significantly lower fibrillin-1 PFFLs in the non-HTN ICCP group compared with both HTN and non-HTN NCCP groups (Fig 2, *A*). Samples from men in the ICCP group had had significantly lower concentrations of fibrillin-1 compared with samples from both men and women in the NCCP group (Fig 2, *B*). Pairwise post hoc testing showed no significant differences in the fibrillin-1 PFFLs between groups with and without a history of diabetes (Fig 2, *C*). Significantly lower fibrillin-2 concentrations were detected between groups when stratified by HTN status, sex, and a history of diabetes (Kruskal-Wallis test; $P = .02$, $P = .034$, and $P = .033$, respectively). Post hoc testing showed significantly lower fibrillin-2 PFFLs in the HTN NICCP samples compared with both groups of NCCP samples. However,

Table IV. Distributions of demographic factors and medical history stratified by aneurysm group

Variable	TAA (n = 27)	TAD (n = 28)	*P* value
Age, years	71 ± 11.0	61 ± 12.7	.0015
Gender			.06
Male	11 (41)	19 (68)	
Female	16 (59)	9 (32)	
BMI, kg/m^2	28.8 ± 6.1	28.8 ± 5.2	.82
Medical history			
Hyperlipidemia	15 (56)	9 (32)	.11
Hypertension	24 (89)	25 (89)	1.0
Diabetes mellitus	2 (7)	1 (4)	.61
COPD	9 (33)	4 (14)	.12
PAD	2 (7)	1 (4)	.61
Stroke	4 (15)	3 (11)	.71
Vascular procedures	14 (52)	21 (75)	.10
Dialysis	0 (0)	1 (4)	1.0
CAD	12 (44)	7 (25)	.16
Angina	6 (22)	5 (18)	.75
MI	5 (19)	2 (7)	.25
Arrhythmia	7 (26)	6 (21)	.76
CHF	2 (7)	4 (14)	.67
Cardiac procedures	11 (41)	16 (57)	.29

BMI, Body mass index; *CAD,* coronary artery disease; *CHF,* congestive heart failure; *COPD,* chronic obstructive pulmonary disease; *MI,* myocardial infarction; *PAD,* peripheral artery disease; *TAA,* thoracic aortic aneurysm; *TAD,* thoracic aortic dissection.
Data presented as mean ± standard deviation or number (%).

Table V. Detectability of plasma fibrillin fragments stratified by dissection status

Variable	TAA (n = 27)	TAD (n = 28)	*P* value
Any fibrillin-1	100 (27)	100 (28)	1.0
B15-HRP26	100 (26)	100 (28)	1.0
B15-HRP78	85 (22)	96 (27)	.18
B15-HRP201	100 (26)	100 (28)	1.0
B201-HRP78	85 (23)	86 (24)	1.0
Any fibrillin-2	78 (21)	96 (27)	.05
B205-HRP143	77 (20)	93 (26)	.14
B72-HRP143	38 (10)	50 (14)	.43

TAA, thoracic aortic aneurysm; *TAD,* thoracic aortic dissection.
Data presented as percentage or (number).

the results of pairwise tests were not significant for sex or diabetes using post hoc testing.

The association of age with fibrillin PFFLs was also assessed. A weak, but statistically significant, negative association of age with fibrillin-1 PFFLs ($\rho = -0.17$; $P = .005$) but not with fibrillin-2 PFFLs (Supplementary Table I) was found. The breakdown of age by quartile also suggested that the fibrillin-1 PFFLs decreased with increasing age (Supplementary Table II).

No significant association was found between the PFFLs and low-density lipoprotein or high-density lipoprotein levels. We also assessed the association of PFFLs with markers of inflammation and CAD risk using the neutrophil/lymphocyte ratio but found no such associations.

Comparison of PFFLs between ED study groups and TAA and TAD groups. Patients with thoracic aortic pathology were separated into those with an aneurysm without dissection (TAA) and those with dissection (TAD), determined by the imaging findings within 1 month of the blood sampling. Six patients within the TAD group had also had aneurysmal dilation of the dissected aorta. The TAD group included 6 patients with acute or subacute aortic dissection and 22 patients with chronic aortic dissection. Patient age, sex, body mass index, and medical history, stratified by dissection status, are listed in Table IV. The patients in both TAA groups were primarily men with an equivalent body mass index. The TAA patients were generally older than the ED patient populations (Table I).

The detectability and maximum fibrillin concentrations were calculated for both aneurysm groups (TAA and TAD). Compared with the ED chest pain groups, the detectability of fibrillin-1 fragments was similar in the aneurysm groups. However, the TAA and TAD groups were more likely to have had detectable fibrillin-2 fragment levels than were the ED group (Table V and Supplementary Table II). The increase in the detectability of fibrillin-2 fragments in those with TAD compared with the TAA group also approached significance ($P = .051$; Table V).

When the maximum fibrillin concentrations were evaluated between the TAD and TAA groups, those with TAD had had higher median and interquartile range values for both fibrillin-1 and fibrillin-2 fragments (Fig 3, *A*). The maximum concentrations for fibrillin-1 were not significantly different between the two groups; however, the maximum concentrations for fibrillin-2 approached significance between the two groups ($P = .06$; Fig 3, *B*).

The fibrillin-2 PFFLs were also compared between the TAA, TAD, and ED groups. The TAD group demonstrated significantly higher fibrillin-2 PFFLs compared with the ED group ($P = .011$); however, no difference was found in the fibrillin-2 PFFLs between the TAA and ED groups ($P = 1.00$).

Fibrillin fragment concentrations measured using different antibody capture-detector pairs. The detectability of the different antibody capture-detector pairs across all study groups is shown in Supplementary Table III. The detectability was significantly different for fibrillin-1 B15-HRP78 ($P = .02$) and for both fibrillin-2 pairs (B205-HRP143, $P < .0001$; B78-HRP-143, $P = .005$). Detectable fibrillin-2 fragment levels were more often found in those with aortic dissection than in all other study groups.

The fibrillin concentrations determined using the different antibody capture-detector pairs are summarized

Table VI. Comparison of fibrillin fragment concentrations within thoracic aortic dissection (TAD) group stratified by type

Fragment	Type A dissection (n = 15)	Type B dissection (n = 13)	*P* value
B15-H201	0.09 (0.06-0.14)	0.09 (0.08-0.13)	.94
B15-H26	0.12 (0.07-0.14)	0.1 (0.07-0.13)	.84
B15-H78	0.13 (0.09-0.23)	0.13 (0.11-0.19)	.84
B201-H78	0.34 (0.04-0.55)	0.09 (0.07-0.64)	.84
B205-H143	0.44 (0.26-0.70)	0.19 (0.09-0.32)	.02
B72-H143	0.02 (0-0.04)	0 (0-0.03)	.11
Maximum fibrillin-1	0.34 (0.13-0.55)	0.21 (0.13-0.64)	.84
Maximum fibrillin-2	0.44 (0.26-0.70)	0.19 (0.09-0.32)	.02

Data presented as median (interquartile range).

in Table III. A comparison of the specific fibrillin fragment concentrations between all five diagnosis groups demonstrated that the B15-HRP26 and B15-HRP78 concentrations were lower in the ICCP group than in the NCCP group. The fibrillin fragment B15-HRP26 concentration was higher in the TAD group than in the ICCP group. In addition, the fibrillin-2 fragment B205-HRP143 levels were increased in the TAD group compared with the ICCP group ($P = .004$) and the NICCP group ($P = .002$) but were not significantly different from those in the NCCP group ($P = .20$). When the PFFLs were compared only among the three ED groups, the B205-HRP143 levels were lower in the ICCP group than in the NCCP group ($P = .025$) and were also lower in the NICCP group than in the NCCP group ($P = .013$). A subgroup analysis of patients in the TAD group demonstrated higher fibrillin-2, fragment B205-HRP143, concentrations in patients with type A TAD compared with patients with type B TAD ($P = .002$; Table VI).

DISCUSSION

Fibrillin-1 and fibrillin-2 are major structural components of the aorta. PFFLs might serve as biomarkers for aortic aneurysm and dissection.[1,2,6] However, because fibrillin-1 and fibrillin-2 are present in nearly all tissues, PFFLs could reflect numerous disease processes, in addition to aortic pathology.[7,8] Therefore, it is important to determine the effects of other clinical conditions on the PFFLs, most importantly those conditions that result in symptoms similar to those from acute aortic syndrome, such as myocardial infarction. Other potential biomarkers for acute aortic syndrome, such as myosin heavy chain and elastin-derived peptides, are also ubiquitously expressed, and, therefore, their levels have been determined in patients presenting with acute myocardial infarction and asymptomatic controls to determine their utility as markers of acute aortic pathology.[7,8]

The results from our study have demonstrated that fibrillin-1 fragments are detectable in all patients presenting to the ED with the chief complaint of chest pain, regardless of the diagnosis. Fibrillin-2 fragments will be detectable in most patients presenting with acute chest pain or aortic pathology but will not be as prevalent as fibrillin-1 fragments. Patients with TAD demonstrated elevated plasma fibrillin-2 fragment levels (B205-HRP143) compared with patients presenting with ischemic or nonischemic cardiac chest pain and increased fibrillin-1 levels (B15-HRP26) compared with patients with ischemic cardiac chest pain. We also found a trend toward increased fibrillin-2 B205-HRP143 in the TAD group compared with the TAA group ($P = .06$). None of the fibrillin-1 or fibrillin-2 antibodies could distinguish between patients with aortic pathology and those with noncardiac chest pain. These findings have shown that the various causes of acute chest pain are associated with differences in fibrillin-1 (B15-HRP26 and B15-HRP78) and fibrillin-2 (B205-HRP143) levels.

A subgroup analysis of the TAD group demonstrated higher fibrillin-2 B205-HRP143 levels in patients with type A aortic dissection than in patients with type B aortic dissection. The mechanism of the different fibrillin-2 fragment levels between type A and type B dissections requires investigation and could include differences in the severity of the aortic injury or regional differences in the amount of fibrillin-2 between aortic segments. Owing to the small number of patients with acute and subacute aortic dissections, we were unable to make any inferences regarding the PFFLs and the acuity of aortic dissection. Similarly, our subgroup analysis of the TAD patients (6 with aneurysmal degeneration and 22 without aneurysmal degeneration) was significantly limited by the small numbers of patients in these groups and did not demonstrate significant differences in any of the PFFL concentrations. Another limitation of our study was the small (n = 6) number of patients with acute or subacute TAD or TAD. Future studies with larger numbers of patients with acute TAD and symptomatic TAD might demonstrate larger differences in PFFLs between patients with acute aortic pathology and patients with other causes of acute chest pain.

Significant PFFL differences were also found within the patients presenting with chest pain of nonaortic etiology. Patients presenting with chest pain due to myocardial

A

PFFL (μg/ml)	ICCP (n=30)	NICCP (n=39)	NCCP (n=194)
Fibrillin-1			
25th percentile	0.09	0.10	0.13
50th percentile	0.13	0.16	0.25
75th percentile	0.68	0.70	0.72
mean	0.50	1.16	1.51
s.d.	0.78	3.49	5.61
Fibrillin-2	ICCP (n=30)	NICCP (n=39)	NCCP (n=194)
25th percentile	0.05	0.04	0.08
50th percentile	0.12	0.10	0.17
75th percentile	0.15	0.16	0.37
mean	0.17	1.73	0.91
s.d.	0.27	8.81	6.24

B

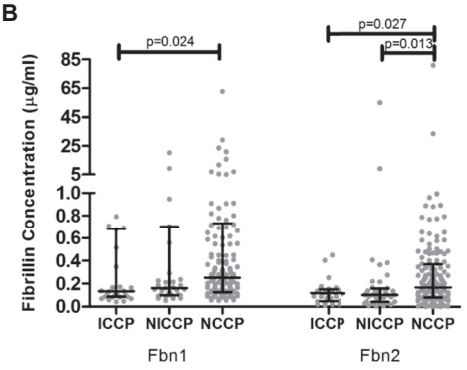

Fig 1. Distribution of fibrillin-1 (*Fbn1*) and fibrillin-2 (*Fbn2*) concentrations stratified by diagnosis group. **A,** Mean and interquartile range for concentrations for fibrillin-1 and fibrillin-2. **B,** The *middle bar* represents the median, and the *whiskers,* the 25th and 75th percentiles. Dunn's post hoc test showed significant differences between ischemic cardiac chest pain (ICCP) and noncardiac chest pain (NCCP) groups for fibrillin-1 ($P = .024$) and fibrillin-2 ($P = .027$) concentrations and between non-ICCP (NICCP) and NCCP groups for fibrillin-2 ($P = .013$) concentrations. No significant difference was found in the fibrillin-1 or fibrillin-2 concentrations between the ICCP and NICCP groups. *PFFL,* Plasma fibrillin fragment level; *s.d.,* standard deviation.

infarction or angina had had lower fibrillin-1 and fibrillin-2 fragment levels than had patients presenting with noncardiac chest pain. Patients with nonischemic causes of cardiac chest pain had had lower fibrillin-2 fragment levels compared with patients with noncardiac chest pain; however, no significant differences were found in fibrillin-1 fragment levels. When the ED chest pain groups were further analyzed according to HTN status and sex, the association between the lower fibrillin-1 levels in the ICCP group than in the NCCP group remained significant for men and for patients without HTN. In contrast, women and patients with HTN showed no significant differences in fibrillin-1 levels between the diagnosis groups. The fibrillin-2 values were also affected by the presence of HTN, with patients with HTN in the NICCP group demonstrating lower fibrillin-2 levels compared with the patients with HTN in the NCCP group. Increasing age was also weakly associated with lower fibrillin-1 levels but not with fibrillin-2 levels. Given the relatively small number of patients in the TAD and TAA groups, a subgroup analysis of the fibrillin levels stratified by sex, age, and medical comorbidities was not performed.

These findings have shown that the various causes of acute chest pain are associated with differences in the fibrillin-1 and fibrillin-2 levels. Specific patient factors and comorbidities such as age, sex, and HTN can also affect the fibrillin fragment levels. Future studies might identify additional factors that affect fibrillin fragment levels; however, our study did not reveal any association between the fibrillin fragment levels and high-density lipoprotein, low-density lipoprotein, diabetes, or the neutrophil/lymphocyte ratio.

The negative association between the fibrillin fragment levels and cardiac disease could also lead to mechanistic insights into the relationship between various cardiac and aortic pathologies. It is possible to hypothesize that systemic atherosclerosis might affect the susceptibility of fibrillin within the medial layer of the arteries to degradation or turnover. Future studies could test this hypothesis by determining the relationship between fibrillin fragment levels and the severity of systemic atherosclerosis. Other studies have suggested a potential relationship between fibrillin-1 genotypes and CAD and aortic disease, with certain fibrillin-1 polymorphisms predictive of aortic stiffness in patients with CAD, but not in healthy individuals.[9–11] Certain fibrillin-1 and -2 polymorphisms have also been associated with an increased risk of myocardial infarction, aortic dissection, and aortic aneurysms.[12–14] Therefore, it is possible that certain fibrillin polymorphisms will also affect circulating PFFLs. Thus, future investigations aimed at developing fibrillin fragment assays as potential biomarkers of aortic disease will need to determine the relationship between fibrillin-1 and fibrillin-2 polymorphisms and PFFLs.

In the present study, we have reported two other potential advances in the use of PFFLs as a potential biomarker. First, the use of HRP-conjugated antibodies improved the detectability of PFFLs compared with previously reported detection rates of alkaline phosphatase-conjugated antibodies,[2] with all patients in the present study having detectable fibrillin-1 PFFLs and most having detectable fibrillin-2 PFFLs. The increased detectability of PFFLs allowed for improved statistical analysis and comparisons between the diagnosis groups. The use of these improved assay methods demonstrated a trend toward increased detectability of fibrillin-2 PFFLs ($P = .051$) and a trend toward a higher fibrillin-2 (B205-HRP143) concentration ($P = .06$) in patients with TAD compared with patients with TAA. These results suggest that fibrillin-2

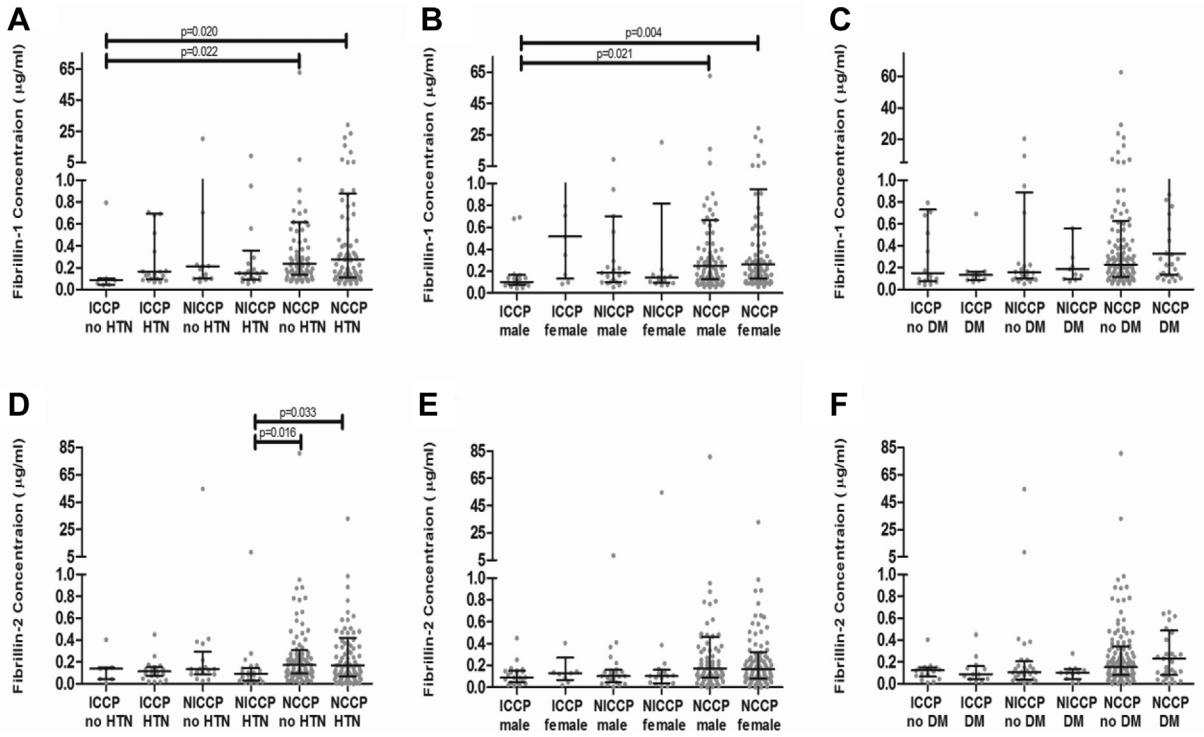

Fig 2. Distributions of fibrillin-1 (**A-C**) and fibrillin-2 (**D-F**) plasma fibrillin fragment level (PFFLs) stratified by chest pain group and hypertension (HTN) status (**A,D**), gender (**B,E**), and history of diabetes mellitus (*DM*; **C,F**). Non-HTN ischemic cardiac chest pain (ICCP) demonstrated significantly lower fibrillin-1 PFFLs compared with both HTN and non-HTN noncardiac chest pain (NCCP) patients ($P = .020$ and $P = .022$, respectively; **A**). ICCP male patients demonstrated lower fibrillin-1 PFFLs compared with NCCP male patients ($P = .021$) and NCCP female patients ($P = .004$; **B**). Non-ICCP (NICCP) patients with HTN demonstrated lower fibrillin-2 PFFLs compared with NCCP patients with HTN ($P = .033$) and NCCP patients without HTN ($P = .016$; **D**). **E,** Gender was not associated with differences in fibrillin-2 PFFLs. **C,F,** DM was not associated with differences in fibrillin-1 or fibrillin-2 PFFLs.

PFFLs should be considered as a potential biomarker for aortic dissection.

Second, the present study investigated six different fibrillin-1 and fibrillin-2 antibody pairs to determine their relative utility in differentiating between patients with different causes of acute chest pain. The association of ischemic cardiac chest pain with lower PFFLs was significant for three of the six antibody pairs—B15-HRP26, B15-HRP78, and B205-HRP143—and the PFFLs detected by the B15-HRP201, B201-HRP78, and B72-HRP143 pairs were not significantly different between the groups. For differentiating between all ED chest pain groups and TAD, fibrillin-2 B205-HRP143 showed the most potential ($P = .0067$). The B205-HRP143 levels also differed significantly between the ICCP and TAD groups ($P = .004$) and between the NICCP and TAD groups ($P = .002$). Fibrillin-2 fragment B205-HRP143 is a relatively large fragment of fibrillin-2 (Supplementary Fig). Elevated fibrillin-2 fragments in TAD patients might occur from systemic pressure within the aortic false lumen that subjects the normally isolated medial layer to hemodynamic forces and inflammatory cells capable of causing fibrillin degradation and elevated circulating PFFLs. Given that atherosclerosis also causes damage to the ECM of arteries, it might seem counterintuitive that circulating PFLLs such as B205-HRP143 and B15-HRP26 would be lower in the ICCP group. The potential mechanisms for the low PFFLs in the ICCP group bear investigation. It might be possible that arterial calcification, ECM cross-linking, and advanced glycosylation end products would render the ECM degradation products less likely to enter the circulation. It is also possible that the ICCP group represents patients with late-stage arterial ECM damage due to prolonged ECM degradation who have relatively less remaining arterial fibrillin and, therefore, lower circulating PFFLs.[15]

The patterns of specific fibrillin fragments associated with ischemic cardiac chest pain compared with acute aortic pathology might provide insights into the pathologic mechanisms of ECM degradation in various cardiovascular diseases, in addition to identifying the most useful biomarkers for these diseases. However, the present study has revealed for the first time, to the best of our knowledge, that increased fibrillin-2 PFFLs are associated with TAD compared with TAA and that increased fibrillin-2 PFFLs are associated with TAD more than

A

PFFL (ug/ml)	TAA (n=27)	TAD (n=28)
Fibrillin-1		
25th percentile	0.11	0.13
50th percentile	0.16	0.25
75th percentile	0.33	0.63
mean	2.02	0.60
s.d.	8.76	0.80
Fibrillin-2	TAA (n=26)	TAD (n=28)
25th percentile	0.03	0.13
50th percentile	0.21	0.30
75th percentile	0.34	0.49
mean	1.59	0.61
s.d.	6.91	1.39

B

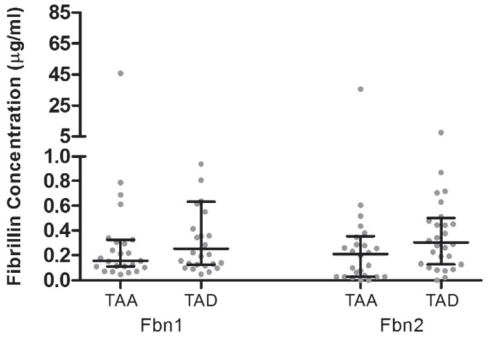

Fig 3. A, Mean and interquartile range for maximum concentrations of fibrillin-1 (*Fbn1*) and fibrillin-2 (*Fbn2*) fragments in aneurysm patients. **B,** Distribution of fibrillin-1 and fibrillin-2 concentrations stratified by diagnosis group. The *middle bar* represents the median, and the *whiskers,* the 25th and 75th percentiles. No significant differences were found between the groups for fibrillin-1 ($P = .17$); however, fibrillin-2 approached significance ($P = .06$; Mann-Whitney *U* test). *TAA,* Thoracic aortic aneurysm; *TAD,* thoracic aortic dissection.

with acute chest pain. Because fibrillin-2 molecules reside within the center of fibrillin microfibrils,[16] the finding of increased fibrillin-2 PFFLs might represent severe degradation of microfibrils. This hypothesis deserves further study.

CONCLUSIONS

The use of PFFLs as a biomarker for acute aortic pathology is still in the early stages. Ongoing data accumulation is needed before determining whether fibrillin fragment levels can be used to identify patients with aortic pathology. Ultimately, PFFLs might prove more useful for monitoring patients with known TAA or TAD as a surrogate marker of ongoing aortic degradation. One particular area of interest will be whether elevated PFFLs will correlate with ongoing aortic dilation in those with TAA and TAD. The purpose of the present study was to investigate other conditions with presentations similar to those of acute aortic syndrome that might also affect the PFFLs and to compare the PFFLs in these conditions with those of TAA patients. Determining the thresholds and critical PFFL ranges will require more data about PFFLs in patients with acute and chronic aortic pathologies, those without aortic pathology, and those with comorbid conditions that could affect the PFFLs. As suggested, both age and sex will be important factors in establishing critical PFFL ranges. Ultimately, a large prospective trial will likely be needed to determine the utility of PFFLs as a potential biomarker for acute aortic pathologies.

We are grateful to Dr Lynn M. Marshall for her overall guidance of the study design, randomization of samples, and oversight of the patient databases. We also thank Nai Saephan, Ribal Salam, Katherine Vu, Rikki Samuel, Tana Repella, Nasibeh Vatankah, Adrienne Fettig, Braden Palanuk, Brady Sanders, and Johnson Vo for assistance with human subjects and with data collection.

AUTHOR CONTRIBUTIONS

Conception and design: EC, MR, GL, GM, CA, LS, AA
Analysis and interpretation: EC, MR, RS, JK, LS, AA
Data collection: DD, TC, KN, CA, AA
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Critical revision of the article: EC, MR, DD, TC, RS, JK, KN, GL, GM, CA, LS, AA
Final approval of the article: EC, MR, DD, TC, RS, JK, KN, GL, GM, CA, LS, AA
Statistical analysis: EC, MR
Obtained funding: JK, LS
Overall responsibility: AA
LS and AA contributed equally to this article and share co-senior authorship.

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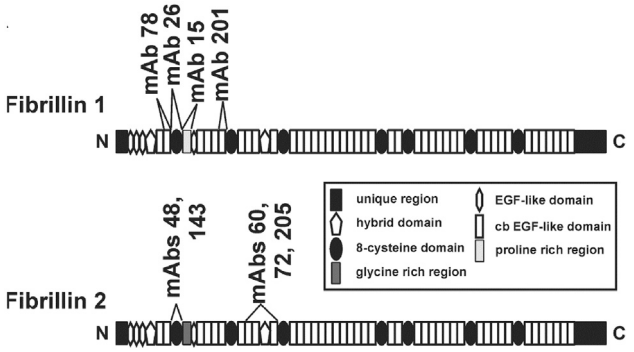
Submitted Apr 26, 2022; accepted Sep 12, 2022.



Supplementary Fig. Epitopes recognized by antibodies used in present study mapped to fibrillin-1 and fibrillin-2 domains. Designations of antibody pairs refer to biotinylated capture antibody and horseradish peroxidase (HRP)-conjugated detector antibody (eg, B15-HRP26). *Cb,* Calcium binding; *EGF,* epidermal growth factor; *mAb,* monoclonal antibody.

Supplementary Table I. Spearman correlation between plasma fibrillin fragment levels (PFFLs) and age[a]

Variable	Spearman rho			
	All samples	ICCP	NICCP	NCCP
Fibrillin-1	−0.17	−0.18	−0.56	−0.04
Fibrillin-2	−0.10	0.14	−0.32	−0.01

ICCP, Ischemic cardiac chest pain; *NCCP,* noncardiac chest pain; *NICCP,* nonischemic cardiac chest pain.
[a]NICCP group showed a weak to moderate association between fibrillin-1 PFFLs and age (as age increased, fibrillin-1 PFFLs decreased); no association found between fibrillin-2 PFFLs and age.

Supplementary Table II. Fibrillin-1 plasma fibrillin fragment levels (PFFLs) for all samples stratified by age quartile[a,b]

Age group, years	Fibrillin-1
<39 (n = 60)	0.29 (0.14-0.71)
39 to <53 (n = 65)	0.23 (0.15-0.78)
53 to <65 (n = 70)	0.18 (0.1-0.69)
≥65 (n = 68)	0.14 (0.09-0.69)

Data presented as median (interquartile range).
[a]As age increased, median fibrillin-1 concentration decreased.
[b]$P = .07$ (Kruskal-Wallis test).

Supplemental Table III. Detectability across diagnosis groups

Variable	ICCP (n = 30)	NICCP 9 (n = 39)	NCCP (n = 194)	TAA (n = 27)	TAD (n = 28)	*P* value
Any fibrillin-1	100 (30)	100 (30)	100 (194)	100 (27)	100 (28)	1.0
B15-HRP26	100 (30)	100 (39)	99 (192)	100 (26)	100 (28)	1.0
B15-HRP78	83 (25)	95 (37)	96 (187)	85 (22)	96 (27)	**.02**
B15-HRP201	100 (30)	100 (39)	99 (192)	100 (26)	100 (28)	1.0
B201-HRP78	80 (24)	87 (34)	93 (180)	85 (23)	86 (24)	.11
Any fibrillin-2	60 (18)	46 (18)	71 (138)	78 (21)	96 (27)	**<.0001**
B205-HRP143	53 (16)	46 (18)	68 (132)	77 (20)	93 (26)	**<.0001**
B72-HRP143	23 (7)	13 (5)	23 (44)	38 (10)	50 (14)	**.005**

ICCP, Ischemic cardiac chest pain; *NCCP,* noncardiac chest pain; *NICCP,* nonischemic cardiac chest pain; *TAA,* thoracic aortic aneurysm; *TAD,* thoracic aortic dissection.
Data presented as percentage (number).
Boldface *P* values represent statistical significance ($P < .05$).