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ORIGINAL ARTICLE

Investigating tissue factor pathway inhibitor and other protease and protease inhibitors and their association with major adverse aortic events in patients with abdominal aortic aneurysm

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Abstract

Background: Abdominal aortic aneurysm (AAA) is characterized by the proteolytic breakdown of the extracellular matrix, leading to dilatation of the aorta and increased risk of rupture. Biomarkers that can predict major adverse aortic events (MAAEs) are needed to risk stratify patients for more rigorous medical treatment and potential earlier surgical intervention.

Objectives: The primary objective was to identify the association between baseline levels of these biomarkers and MAAEs over a period of 5 years.

Methods: Baseline levels of 3 proteases (matrix metalloproteinases 7, 8, and 10) and 3 protease inhibitors (tissue factor pathway inhibitor [TFPI], SerpinA12, SerpinB3) were investigated. Plasma levels of these biomarkers were quantified in 134 patients with AAA and 134 matched controls. Patients were followed for a 5-year period during which MAAEs were documented. The association between these markers and MAAEs was evaluated using Cox regression and Kaplan–Meier survival curves.

Results: TFPI was significantly elevated in patients with AAA and significantly associated with MAAE during the 5-year period (hazard ratio, 1.52; 95% CI, 1.15-2.01; P = .003) after adjusting for covariates. Kaplan–Meier survival analyses demonstrated that patients in the high TFPI group (defined as plasma levels >25.961 ng/mL) had significantly reduced freedom from the need for aortic repair and MAAEs.

Conclusion: These findings suggest that TFPI may serve as a valuable prognostic marker for the risk of MAAEs within 5 years in patients with AAA, potentially offering new tools for the medical management of patients with AAA.

KEYWORDS
abdominal aortic aneurysm, aneurysm, biomarkers, major adverse aortic events, prognosis, vascular

Essentials

- Abdominal aortic aneurysm (AAA) can lead to aortic rupture and increased risk of death.
- Biomarkers are needed for prognostication in patients with AAA.
- Tissue factor pathway inhibitor (TFPI) is associated with major adverse aortic events.
- TFPI should be investigated for its clinical use as a prognostic marker in patients with AAA.

1 | INTRODUCTION

Abdominal aortic aneurysm (AAA) is a disease of the lower abdomen that is characterized by the loss of the elasticity within the aortic wall, leading to dilatation of the artery and an increased risk of rupture, a catastrophic and deadly consequence of the disease [1]. This loss of elasticity has been attributed to increased inflammation within the medial and intimal layers, reduction in matrix-synthesizing medial smooth muscle cells, and excessive extracellular matrix (ECM) degradation by proteolytic enzymes [2]. Several studies have proposed biomarkers for AAA screening [3] and prognosis of AAA [4]; however, markers for the prognostication of AAA-specific events that are clinically relevant and widely applied are lacking, specifically within proteolytic enzymes that may be directly or indirectly involved in the enzymatic breakdown of the ECM. Previous studies have investigated 2 proteolytic enzymes as prognostic markers for AAA, matrix metalloproteinase-9 (MMP-9) and elastase, noting both biomarkers correlate with AAA growth rate [5–7]. To our knowledge, no study has followed patients long term or investigated baseline levels of proteolytic enzymes and inhibitors and their ability to predict long-term aortic-related events in patients with AAA. In this study, we investigated several different proteases and protease inhibitors and their relationship to AAA and AAA-related outcomes.

MMPs are proteinases that have well-known functions in the breakdown of several ECM proteins [8]. Specifically, MMP-7 is primarily known for its proteolytic cleavage of fibronectin, which plays a critical role in the structural stability and function of the ECM [9,10]. It has also been demonstrated to mediate the release of the cytokine tissue necrosis factor from macrophages, modulating the immune response [11]. MMP-8 is a member of the same protein family and hydrolyzes collagens, gelatin, elastins, matrix glycoprotein, and other ECM proteins to aid in angiogenesis, vascular remodeling, and inflammation [12]. Similarly, MMP-10 has a wide range of ECM substrates including proteoglycans, laminin, gelatins, fibronectin, entactin, and collagens particular to angiogenesis [13].

Tissue factor pathway inhibitor (TFPI or TFPI-1) is a serine protease inhibitor that is primarily known for its function as an inhibitor of coagulation. It achieves this by inhibiting both factor Xa, as well as the tissue factor–factor VIIa complex [14]. Although TFPI primarily functions in regulating coagulation, a homologous protein with structural similarities, TFPI-2, is known to regulate MMPs and ECM remodeling [15]. Whether TFPI-1 functions in modulating the ECM is unknown. Patients with AAA often have thrombus formation within aneurysm sac walls, and TFPI may also be elevated in this patient population for the regulation of this hypercoagulable state.

SerpinA12 (also known as vaspin) and SerpinB3 (also known as squamous cell carcinoma antigen 1 [SCCA-1]) are 2 serine protease inhibitors that belong to the Serpin family. SerpinA12 has several functions including the regulation of cell proliferation and apoptosis, as well as inhibiting the activity of proinflammatory cytokines such as tissue necrosis factor and interleukin-6 [16]. AAA progression is characterized by the increase in damage to the endothelium within the aorta and dysregulation of the inflammatory response, and hence SerpinA12 could be a marker of AAA progression. Similarly, SerpinB3 is known to be a regulator of apoptosis; however, it has a well-established role in the regulation of the ECM. Studies have demonstrated that overexpression of SerpinB3 can lead to the upregulation of MMPs, including MMP-9, a known marker of cardiovascular disease, AAA growth rate, and the risk of endoleak post endovascular aortic repair and hence may predict adverse outcomes in patients with AAA [4,17].

No previous research has investigated MMP-7, MMP-8, MMP-10, TFPI, SerpinA12, and SerpinB3 as potential prognostic markers for AAA-related outcomes, despite their potential involvement with the disease process. Proteolytic, inflammatory, and coagulative pathologies are well-known drivers of AAA formation. These markers were selected, specifically MMPs and serine protease inhibitors such as TFPI, SerpinA12, and SerpinB13, due to their known roles in vascular remodeling and inflammation. SerpinA12 and SerpinB13 were included because serine protease inhibitors in this family have been shown to regulate inflammatory processes in the vascular environment through protease inhibition, contributing to aneurysm development and growth [18]. These markers were also chosen due to the ability to accurately detect them within plasma. Some other markers, including tissue inhibitors of metalloproteinases, were not included because of the findings of previous studies [4–6]. In this study, our primary objective was to investigate the association between the plasma levels of these biomarkers at baseline and the 5-year risk of major adverse aortic events (MAAEs) in patients with AAA.

2 | METHODS

2.1 | Patient recruitment

Consecutive patients were recruited from St. Michael's Hospital vascular surgery ambulatory clinics from May 2017 to July 2019. Each patient underwent a physical examination and evaluation by a certified vascular specialist after undergoing ultrasound, computed tomography angiography, or magnetic resonance angiography imaging

of the aorta. The aortic diameter of the abdominal aorta was recorded. The inclusion criteria for the AAA group were a dilatation of the suprarenal or infrarenal aorta of ≥3 cm on imaging and 18 years of age or older. Inclusion criteria for the matched control patients were suprarenal or infrarenal aorta measurements <3 cm and 18 years of age or older. Patients were excluded if they had an AAA diameter >5.5 cm, presented with a ruptured AAA, or symptoms that were associated with the AAA (abdominal or ischemic pain).

2.2 | Clinical characteristics and plasma biomarker quantification

Age, sex, history of hypertension (patients taking medication to lower blood pressure, with a systolic blood pressure of >130 mmHg and a diastolic blood pressure of >80 mmHg), hypercholesterolemia (people on lipid-lowering therapy with triglyceride levels >1.7 mmol/L or total cholesterol >5.2 mmol/L), diabetes mellitus (patients with HbA1c >6.5%), coronary artery disease and/or congestive heart failure (defined as a diagnosis documented within their clinical chart by a cardiologist), and smoking status (current, past, or never) were gathered at baseline [19].

Blood was drawn from the antecubital vein into 3.2% sodium citrate tubes at baseline. Plasma samples were collected within 1 hour of blood draw by centrifugation at 1000 × *g* for 10 minutes at 4 °C and stored at −80 °C until analysis. LUMINEX assay (Bio-Techne) was used to quantify plasma levels of MMP-7, MMP-8, MMP-10, free TFPI, SerpinA12, and SerpinB3 in duplicate, as per manufacturer's instructions.

2.3 | Follow-up visits and outcomes

Patients were followed up at 6 or 12 months over a period of 5 years based on guidelines provided by the Society for Vascular Surgery [20]. Patients underwent a follow-up physical examination and evaluation by their certified vascular specialist after undergoing ultrasound, computed tomography angiography, or magnetic resonance angiography imaging of the aorta. Measurement of the abdominal aorta was recorded. Patients were questioned about recent medical history, and any incidence of need for elective or emergent aortic repair (open AAA repair or endovascular aortic repair), rapid aortic growth, and aneurysmal ruptures was recorded. MAAE was defined as a composite of all 3 events. Rapid aortic growth was defined as aortic growth ≥0.5 cm in 6 months or ≥1 cm in 1 year, as per previous studies [21]. Patients were contacted by telephone after the 5-year period for final data collection.

2.4 | Statistical analysis

Demographics and clinical characteristics are reported as mean ± SD for continuous variables and number of patients (percentage overall) for categorical variables. Independent *t* tests were used to test differences between continuous variables that were normally distributed. For non-normally distributed characteristics, Mann–Whitney U-test was used. Chi-squared tests were used to test the differences between categorical variables.

For this pilot study, the primary objective was to investigate the association between biomarker levels and 5-year outcomes of MAAE in patients with AAA. The secondary objective was to investigate and compare levels of biomarkers between patients with AAA and matched controls, as well as between AAA patients who had an MAAE and those who had not. We also sought to investigate the association between biomarker levels and AAA diameter at baseline. The final secondary objective was to determine if there was a significant difference in freedom from MAAEs when stratifying patients based on biomarker levels.

For our primary objective, Cox proportional hazard regression analysis was conducted on *z*-score normalized biomarkers for each MAAE and adjusted for age, sex, and history of hypertension, hypercholesterolemia, diabetes mellitus, smoking status (current, previous, never), congestive heart failure, and coronary artery disease, and hazard ratios (HRs) and 95% CIs were calculated. Each variable in the regression model was tested to ensure that the proportional hazards assumption was met. Categorical variables were tested by assessing parallelism of log(−log) plots. For both categorical and continuous variables, interaction terms were created with each variable and time. The absence of a significant interactive term (β coefficient) was defined as meeting the proportional hazards assumption. The model was created using *z*-score normalized levels of each biomarker and adjusted for age, sex, and history of hypertension, hypercholesterolemia, smoking status, congestive heart failure, and coronary artery disease as covariates without any interactive terms.

For our secondary objective, Mann–Whitney U-tests were used to compare biomarker levels between AAA and matched controls, as well as AAA patients who suffered from an MAAE vs those who did not. Simple linear regression was used to determine the association between biomarker levels at baseline and baseline AAA diameter. Receiver operating characteristic analysis was conducted and Youden index calculated to choose the best cutoff value for MAAE, and patients were split into groups by high or low biomarker levels. Freedom from outcomes (need for aortic repair, rapid aortic growth, aortic rupture, and MAAEs) was determined by Kaplan–Meier curves, and differences between the high and low biomarker groups were compared by a log-rank test.

If a full 5-year follow-up was not completed, or patients were not available for a telephone follow-up after 5 years, patients were deemed censored at the time of their last follow-up contact. These patients were included in both the Kaplan–Meier survival analysis and the Cox proportional hazards regression model as censored observations. Statistical significance was set as $P < .05$. All tests were completed using SPSS Version 29.0.0.0 (241) and GraphPad Prism Version 10.1.0 (264).

2.5 | Ethics

Protocols for the study were approved by the Unity Health Network Research Ethics Board. All patients provided written informed consent before enrollment. Our protocols followed the World Medical Association Declaration of Helsinki [22].

3 | RESULTS

3.1 | Demographics and clinical characteristics

A total of 268 patients were recruited to the study, consisting of 134 patients with AAA and 134 matched controls without AAA. There were no statistical differences between any of the demographics and clinical characteristics. Patients with AAA had a significantly higher mean abdominal aortic diameter than controls, with mean aneurysm sizes of 4.10 ± 0.87 and 2.01 ± 0.35, respectively ($P < .0001$). The mean age of patients with AAA was 72 ± 11 years, with 81% of patients being male. There was a high rate of cardiovascular risk factors such as hypertension (69% of patients in the AAA group and 63% in controls, $P = .30$), hypercholesterolemia (79% of patients in the AAA group and 77% in controls, $P = .55$), and smoking (Table 1).

TABLE 1 Demographics and clinical characteristics of patients with AAA ($n = 134$) and matched controls without AAA ($n = 134$).

Characteristic	AAA ($n = 134$)	Controls ($n = 134$)	P
Age (y)	71 ± 10	73 ± 11	.79
Aneurysm diameter at baseline (cm)	**2.01 ± 0.35**	**4.10 ± 0.87**	**<.0001**
Sex (male)	113 (84)	103 (77)	.12
Hypertension	93 (69)	84 (63)	.30
Hypercholesterolemia	108 (81)	103 (77)	.55
Diabetes mellitus	30 (22)	19 (14)	.11
Smoking status			
Current	36 (27)	40 (30)	.69
Past	80 (60)	76 (57)	.71
Never	18 (13)	18 (13)	>.99
Congestive heart failure	7 (5)	4 (3)	.54
Coronary artery disease	57 (43)	48 (36)	.32
Medication			
Statin	103 (77)	96 (72)	.40
ACE/ARBi	77 (57)	68 (51)	.33
β-blocker	44 (33)	39 (29)	.60
Calcium channel blocker	34 (25)	30 (22)	.67
Oral antihyperglycemic	19 (14)	11 (8)	.17
Insulin	4 (3)	3 (2)	>.99
Antiplatelet	90 (67)	87 (65)	.80
Anticoagulant	25 (19)	24 (18)	>.99
Dual antiplatelet and anticoagulant	12 (9)	6 (4)	.22

Bolded text indicates variables with statistically significant differences.
AAA, abdominal aortic aneurysm; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker.

3.2 | Biomarker levels in patients with AAA vs controls

Of the 6 biomarkers analyzed, only TFPI was significantly elevated in patients with AAA relative to matched controls. Specifically, patients with AAA had a mean TFPI plasma level of 14.072 ± 4.618 ng/mL vs 21.19 ± 9.711 ng/mL in control patients without AAA ($P < .001$). No other biomarkers were significantly different between patients with AAA and controls (Table 2).

3.3 | Associations between biomarker levels and MAAEs

As none of the patients within the control group had an MAAE, the following analysis was conducted only in the group of patients with AAA. In the 134 patients with AAA, there were 46 patients (34%) who underwent elective or emergent aortic repair. Within the 5-year period, 24 (18%) patients had rapid aortic growth, and 1 patient had an aneurysm rupture that resulted in death (Table 3).

Patients with AAA ($n = 134$) were then split into those who had an MAAE ($n = 53$) and those who had not ($n = 81$). Demographics and clinical characteristics are shown in Table 4. Again, there were no statistically significant differences in demographics or clinical characteristics between patients who had an MAAE vs those who had not, except for AAA size at baseline. Patients with AAA who experienced an MAAE had significantly larger aneurysm diameter at baseline

TABLE 2 Biomarker levels in plasma of patients with AAA ($n = 134$) and matched controls without AAA ($n = 134$).

Biomarker	AAA ($n = 134$)	Controls ($n = 134$)	P
MMP-7 (ng/mL)	4.127 ± 2.211	4.537 ± 2.427	.21
MMP-8 (ng/mL)	261.774 ± 321.633	320.796 ± 339.671	.10
TFPI (ng/mL)	**14.072 ± 4.618**	**21.19 ± 9.711**	**<.001**
MMP-10 (pg/mL)	585.494 ± 301.155	613.077 ± 265.554	.13
Serpin A12 (pg/mL)	72.961 ± 52.555	77.635 ± 54.521	.48
Serpin B3/ SCCA1 (pg/mL)	180.778 ± 140.324	167.05 ± 132.919	.48

Bolded text indicates variables with statistically significant differences.
AAA, abdominal aortic aneurysm; MMP, matrix metalloproteinase; SCCA1, squamous cell carcinoma antigen 1; TFPI, tissue factor pathway inhibitor.

TABLE 3 Rates of MAAEs in patients with AAA (*N* = 134) within a 5-year period.

Adverse event	Patients with AAA (*N* = 134)
Need for aortic repair	46 (34)
Rapid AAA growth	24 (18)
Aneurysm rupture	1 (0.7)
MAAEs	53 (40)

AAA, abdominal aortic aneurysm; MAAE, major adverse aortic event.

(4.60 ± 0.87 cm) than who did not (3.76 ± 0.69 cm), with $P < .001$ (Table 4).

Biomarker analysis demonstrated that TFPI was significantly elevated in patients with AAA who had an MAAE vs those who had not. AAA patients with an MAAE, when compared to those without an MAAE, had mean TFPI levels of 24.119 ± 10.933 ng/mL vs 20.499 ± 10.515 ng/mL ($P = .03$), respectively (Supplementary Table 1). Due to the significantly elevated levels of TFPI in patients with AAA who had an MAAE, we focused on this protein and its association with MAAE in patients with AAA over a 5-year period. Cox proportional hazard regression analysis and Kaplan–Meier analysis were then conducted to determine associations between TFPI and the risk of MAAE over this 5-year period. The mean follow-up time was 55 ± 13 months, with 109 patients completing the full 5-year follow-up, with 17 deaths and 8 censored patients.

3.3.1 | TFPI levels and MAAEs in patients with AAA

Univariate Cox regression analysis demonstrated that TFPI was significantly associated with both need for aortic repair (HR, 1.69; 95% CI, 1.29-2.20; $P < .001$), and MAAE (HR, 1.37; 95% CI, 1.06-1.77; $P = .02$). Multivariate Cox regression analysis was then performed, adjusting for the following covariates: age, sex, smoking status, and history of hypertension, hypercholesterolemia, diabetes mellitus, congestive heart failure, and coronary artery disease. After adjusting for covariates, TFPI remained significantly associated with an increased risk of the need for aortic repair, with an HR of 1.88 (95% CI, 1.38-2.56; $P < .001$), indicating that with every 1-unit increase in *z*-score-normalized TFPI, there was an 88.2% increase in the risk of

TABLE 4 Demographics and clinical characteristics of patients with AAA (*N* = 134) who had an MAAE (*n* = 53) vs those who had not (*n* = 81).

Characteristic	Overall (*N* = 134)	MAAE (*n* = 53)	No MAAE (*n* = 81)	*P*
Age (y)	73 ± 11	72 ± 9	73 ± 12	.17
AAA diameter at baseline (cm)	**3.10 ± 1.21**	**4.60 ± 0.87**	**3.76 ± 0.69**	**<.001**
Sex (male)	113 (42)	45 (85)	68 (84)	>.99
Hypertension	93 (35)	35 (66)	58 (72)	.57
Hypercholesterolemia	108 (40)	44 (83)	64 (79)	.66
Diabetes mellitus	30 (11)	12 (23)	18 (22)	>.99
Smoking status				
Current	36 (13)	12 (23)	24 (30)	.43
Past	80 (30)	33 (62)	47 (58)	.72
Never	18 (7)	8 (15)	10 (12)	.80
Congestive heart failure	7 (3)	2 (4)	5 (6)	.70
Coronary artery disease	57 (21)	21 (40)	36 (44)	.60
Medication				
Statin	103 (38)	44 (83)	59 (73)	.21
ACEi/ARB	77 (29)	33 (62)	44 (54)	.38
β-blocker	44 (16)	16 (30)	28 (35)	.71
Calcium channel blocker	34 (13)	10 (19)	24 (30)	.22
Oral antihyperglycemic	19 (7)	5 (9)	14 (17)	.33
Insulin	4 (1)	2 (4)	2 (2)	>.99
Antiplatelet	90 (67)	38 (72)	52 (64)	.45
Anticoagulant	25 (19)	7 (13)	18 (22)	.26
Dual antiplatelet and anticoagulant	12 (9)	2 (4)	10 (12)	.12

Bolded text indicates variables with statistically significant differences.

AAA, abdominal aortic aneurysm; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; MAAE, major adverse aortic event.

TABLE 5 Association between tissue factor pathway inhibitor and MAAE by Cox proportional hazard regression analysis in patients with AAA after 5-year follow-up.

Adverse event	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Need for aortic repair	**1.69 (1.30-2.20)**	**<.001**	**1.882 (1.384-2.560)**	**<.001**
Rapid AAA growth	1.06 (0.70-1.59)	.80	1.068 (0.695-1.640)	.76
MAAE	**1.37 (1.06-1.77)**	**.02**	**1.528 (1.154-2.022)**	**.003**

P value < .05 was considered statistically significant and are bolded.
AAA, abdominal aortic aneurysm; HR, hazard ratio; MAAE, major adverse aortic event.

needing an aortic repair in the 5-year period. For MAAE, the HR was 1.53 (95% CI, 1.15-2.02, $P = .003$), indicating with every 1-unit increase in *z*-score-normalized TFPI, there was a 52.8% increased risk of an MAAE in the 5-year period (Table 5 and Figure 1). No other covariates were significantly associated with MAAEs (Supplementary Table 2).

3.3.2 | Risk stratification of patients with AAA based on TFPI levels

Patients were then split into high vs low levels of TFPI using receiver operating characteristic analysis. Patients were considered as having high TFPI levels if plasma TFPI was >25.961 ng/mL. Patients in the high TFPI group had significantly higher baseline AAA diameters (4.61 ± 0.92 vs 3.94 ± 0.73; $P < .001$) and were more likely to have congestive heart failure (13% vs 2%; $P = .02$) than patients in the low TFPI group, respectively. No other demographic or clinical characteristics were significantly different between the 2 groups (Table 6).

Patients in the high TFPI group also had significantly higher rates of the need for surgical intervention (63% vs 23%; $P < .001$) and MAAEs (82% vs 23%; $P < .001$) than patients with low TFPI levels. There was no significant difference between the 2 groups in rates of rapid AAA growth (Table 7).

Kaplan–Meier analysis demonstrated that patients in the high TFPI group had significantly lower freedom from need for aortic repair than those in the low TFPI group, with only 39% of patients in the high group remaining free from the need for surgical repair compared with 78% in the low TFPI group (log rank, 22.2; $P < .0001$). Similarly, regarding MAAEs, patients in the high TFPI group had significantly lower freedom from MAAEs, with 53% of patients remaining free from an MAAE compared with the low TFPI group, in which 78% remained free from MAAEs (log rank, 22.2; $P < .0001$) (Figure 2).

3.4 | Association between biomarker levels and aneurysm size at baseline

To investigate the link between biomarker levels and AAA, a simple linear regression was conducted to determine the association between plasma biomarker levels at baseline and AAA diameter at baseline in all patients. Of the 7 markers, TFPI was linearly associated with aneurysm size. Specifically, with every 1-unit increase in *z*-score-normalized TFPI level, there was an increase in aneurysm size by 0.251 cm (95% CI, 0.07-0.36; $P = .004$). No other biomarker was significantly linearly associated with aneurysm size at baseline (Table 8).

4 | DISCUSSION

The results of this study suggest that TFPI may act as a potential prognostic biomarker of MAAE. TFPI was significantly elevated in patients with AAA when compared with matched controls and was

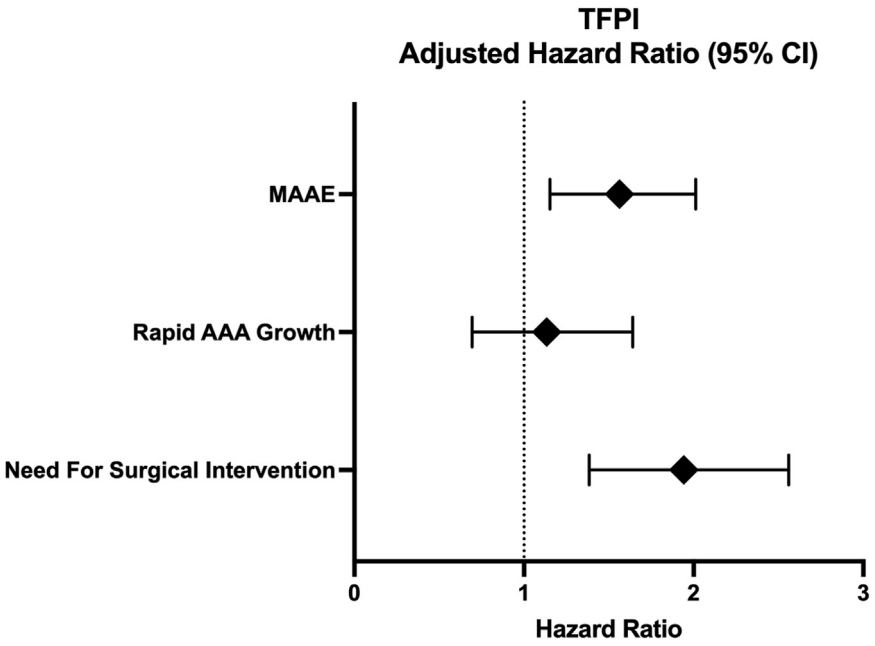

FIGURE 1 Adjusted hazard ratios (HR) and 95% CIs for major adverse aortic events (MAAEs) for every 1-unit increase in *z*-score-normalized tissue factor pathway inhibitor (TFPI). Analysis was adjusted for age, sex, smoking status (current, past, never), and history of hypertension, hypercholesterolemia, diabetes, congestive heart failure, and coronary artery disease. AAA, abdominal aortic aneurysm.

TABLE 6 Demographics and clinical characteristics of patients with AAA (*N* = 134) with low (*n* = 96) vs high (*n* = 38) TFPI levels based on receiver operating characteristic analysis.

Characteristic	Low TFPI (*n* = 96)	High TFPI (*n* = 38)	*P*
Age (y)	73 ± 12	73 ± 9	.90
AAA diameter at baseline (cm)	**3.94 ± 0.73**	**4.61 ± 0.92**	**<.001**
Sex (male)	81 (84)	32 (84)	>.99
Hypertension	70 (73)	23 (61)	.21
Hypercholesterolemia	78 (81)	30 (79)	.81
Diabetes mellitus	21 (22)	9 (24)	.82
Smoking status			
Current	25 (26)	11 (29)	.830
Past	55 (57)	25 (66)	.44
Never	16 (17)	2 (5)	.10
Congestive heart failure	**2 (2)**	**5 (13)**	**.02**
Coronary artery disease	41 (43)	16 (42)	>.99
Medication			
Statin	74 (77)	29 (76)	>.99
ACEi/ARB	58 (60)	19 (50)	.33
β-blocker	26 (27)	12 (32)	>.99
Calcium channel blocker	26 (27)	8 (21)	.52
Oral antihyperglycemic	11 (11)	7 (18)	.40
Insulin	3 (3)	1 (3)	>.99
Antiplatelet	66 (69)	26 (68)	.54
Anticoagulant	20 (21)	5 (13)	.46
Dual antiplatelet and anticoagulant	11 (11)	1 (3)	.18

A patient was considered to have high TFPI if their baseline TFPI level was >25.961 ng/mL. Bolded text indicates variables with statistically significant differences.

AAA, abdominal aortic aneurysm; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; TFPI, tissue factor pathway inhibitor.

TABLE 7 Major adverse cardiovascular events in patients split into 2 groups (High vs Low) by median HE4 level (9.338 ng/mL).

Adverse event	Overall (*N* = 134)	High TFPI (*n* = 38)	Low TFPI (*n* = 96)	*P*
Need for aortic repair	**46 (34)**	**24 (63)**	**22 (23)**	**<.001**
Rapid AAA growth	24 (18)	17 (45)	7 (7)	.92
MAAE	**53 (40)**	**31 (82)**	**22 (23)**	**<.001**

Comparisons between the High HE4 vs Low HE4 group with *P* value < .05 were considered as statistically significant and are bolded.

AAA, abdominal aortic aneurysm; MAAE, major adverse aortic event.

linearly associated with AAA diameter at baseline. Of 6 six markers tested, only TFPI was significantly elevated in AAA patients who experienced an MAAE compared with those without an MAAE. TFPI was significantly associated with the need for aortic repair and MAAEs. AAA patients with higher plasma TFPI levels were significantly less likely to be free from the need for aortic repair and MAAEs in the 5-year period.

AAA is a progressive cardiovascular disease that is characterized by the proteolytic breakdown of the ECM within the media of the abdominal aortic artery, leading to structural deficiencies that increase the risk of rupture [23]. Aortic ruptures, though rare with monitoring and early intervention, have a mortality of over 80%. Recent studies have demonstrated that early screening in patients >65 years of age can reduce the rates of death by >81% [24]. Determining patients who are at risk of adverse aortic events may be beneficial to provide these patients with more strict medical management, earlier interventions, or more rigorous screening. There are no current studies investigating protease and protease inhibitors and their association with long-term AAA-related outcomes.

In this study, TFPI, a serine protease inhibitor, was associated with the need for AAA repair and MAAEs in patients with AAA, and those with higher levels of plasma TFPI were less likely to have freedom from the need of aortic repair and MAAEs in the 5-year period. It is well known that patients with AAA are at an increased risk of thrombus formation both in the blood and within the intraluminal wall of the aorta, known as intraluminal thrombus (ILT) [25]. Damage to the aorta can lead to the exposure of tissue factor and the initiation of coagulation [26]. TFPI release is enhanced by tissue factor and hence, higher levels of TFPI in patients with MAAEs may be related to a compensatory mechanism occurring in patients with elevated ILT. ILT is associated with increased adverse aortic events and known to increase AAA growth rates [27]. However, TFPI may not only be influencing coagulation but may also affect ECM proteins as well. Whether TFPI-1 can directly lead to ECM degeneration is unknown; however, TFPI-2, a homolog of TFPI-1, is known to regulate MMPs and modulate ECM degradation [28]. Elevated TFPI levels being associated with MAAEs likely reflects a complex interplay of coagulation, inflammation, endothelial dysfunction, and ECM modulation, acting to regulate excessive inflammation and coagulation in response to the pathological processes of AAA.

Other than surgical interventions, there are currently no available therapeutic treatments to slow the progression of AAA or reduce the risk of MAAE. AAA is managed through cardiovascular risk reduction, as well as monitoring until the risk of rupture becomes significant (when abdominal aortic diameter becomes ≥5.5 cm) [20]. In this study, we showed the significant associations between TFPI and MAAEs in patients with AAA. Although not investigated directly in this study, targeting TFPI through medical therapy may serve as a new therapeutic method to prevent the complications associated with AAA. Further research into these plasma proteins and the ability to medically target them could offer a new avenue for managing AAA and reducing the risk of adverse outcomes.

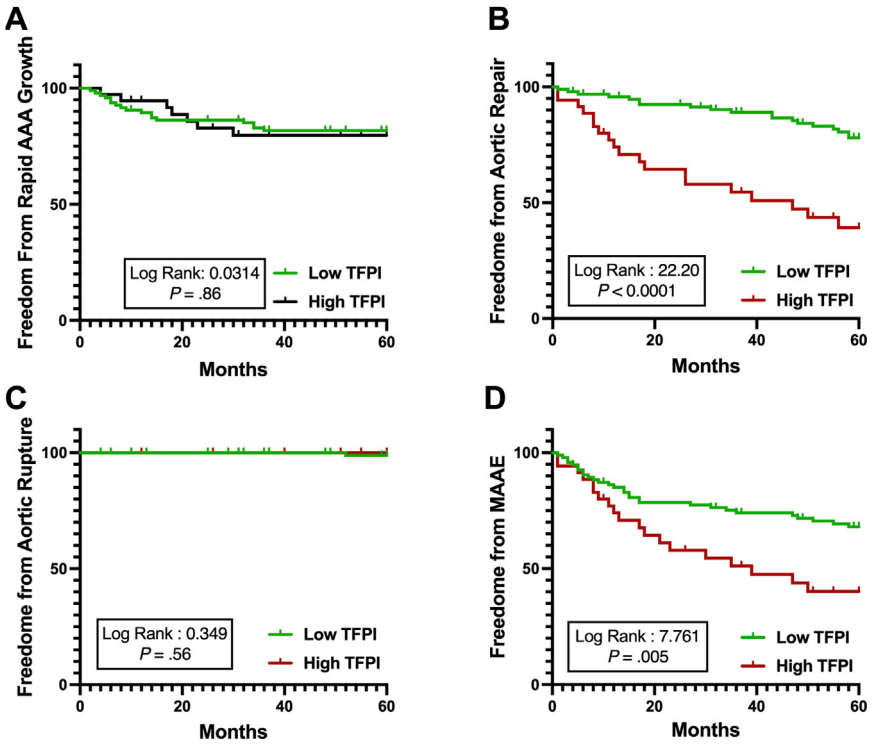

FIGURE 2 Kaplan–Meier analysis of freedom from major adverse aortic events (MAAEs) in patients with abdominal aortic aneurysm (AAA) with low tissue factor pathway inhibitor (TFPI) (n = 96) vs high TFPI (n = 38) for, (A) rapid AAA growth, (B) need for aortic repair, (C) aortic rupture, and (D) MAAE.

Prognostic markers may be beneficial as screening tools for patients with AAA and for risk stratification purposes. The current guideline for screening patients with diagnosed AAA is 1 to 2 years; however, some patients at risk of MAAEs may benefit from more frequent follow-up appointments. Also, those who are at increased risk of MAAEs could be offered more rigorous risk reduction therapy and earlier surgical intervention to offset the risk of these adverse events.

There are some limitations to our study. First, this was a single-center observational study with a relatively small sample size. A few clinical characteristics that could potentially have influenced the biomarker level association with MAAEs, such as race, body mass index, and socioeconomic status, were not included in the analysis. Race has been demonstrated to play a role in AAA diagnosis and hence, its inclusion should be considered in future studies. Due to the observational nature of this study, residual confounding and selection bias may not have been entirely ruled out. Tissue factor and factor Xa levels and ILT were not investigated in this study. Future analysis of TFPI in AAA patients with and without ILT may help to further determine the mechanism for increased TFPI levels in patients with AAA and those who have experienced an MAAE. Second, those patients who did not have a follow-up scheduled were contacted by telephone to determine the occurrence of any events, which may have led to recall bias. Tissue inhibitors of metalloproteinases, as well as KLK7 and cathepsin L, which are the targets of the Serpins investigated in this study, were not measured. In future studies, investigating these serine proteases may help further elucidate the underlying mechanisms of our results.

TABLE 8 Linear regression results for association between z-score normalized biomarkers levels and AAA size at baseline in patients with AAA.

Biomarker	Coefficient (β)	95% CI	P
MMP-7	0.106	−0.04, 2.55	.16
MMP-8	−0.029	−0.18, 0.12	.70
TFPI	**0.251**	**0.07, 0.36**	**.004**
MMP-10	0.043	−0.11, 0.19	.57
Serpin A12	−0.094	−0.24, 0.05	.22
Serpin B3/SCCA1	−0.013	−0.16, 0.14	.86

Bolded text indiciates variables that are statistically significant.

AAA, abdominal aortic aneurysm; MMP, matrix metalloproteinase; SCCA1, squamous cell carcinoma antigen 1; TFPI, tissue factor pathway inhibitor.

In conclusion, TFPI was significantly associated with MAAEs and could potentially be used as a prognostic biomarker for MAAEs. Further research is required in a larger sample of patients to further elucidate the relationship between TFPI levels and MAAEs in patients with AAA, as well as to investigate the potential of targeting this marker for therapeutic intervention to reduce MAAEs.

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AUTHOR CONTRIBUTIONS

Conceptualization: H.K., M.Q.; methodology: H.K., A.Z., F.S., M.Q.; formal analysis: H.K., A.Z., F.S., G.S., M.M., M.Q.; data interpretation: H.K., A.Z., F.S., G.S., M.M., M.Q.; writing: H.K., A.Z., F.S., G.S., M.M., M.Q. All

authors have read and approved the final manuscript and agree to the published version of the article.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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REFERENCES

[1] Jeanmonod D, Yelamanchili VS, Jeanmonod R. Abdominal aortic aneurysm rupture. StatPearls. Treasure Island, FL. StatPearls Publishing; 2024.

[2] Shah PK. Inflammation, metalloproteinases, and increased proteolysis: an emerging pathophysiological paradigm in aortic aneurysm. *Circulation*. 1997;96:2115–7. https://doi.org/10.1161/01.CIR.96.7.2115

[3] Stather PW, Sidloff DA, Dattani N, Gokani VJ, Choke E, Sayers RD, et al. Meta-analysis and meta-regression analysis of biomarkers for abdominal aortic aneurysm. *Br J Surg*. 2014;101:1358–72. https://doi.org/10.1002/bjs.9593

[4] Khan H, Abu-Raisi M, Feasson M, Shaikh F, Saposnik G, Mamdani M, et al. Current prognostic biomarkers for abdominal aortic aneurysm: a comprehensive scoping review of the literature. *Biomolecules*. 2024;14:661. https://doi.org/10.3390/biom14060661

[5] Georgiadis GS, Antoniou GA, Argyriou C, Schoretsanitis N, Nikolopoulos E, Kapoulas K, et al. Correlation of baseline plasma and inguinal connective tissue metalloproteinases and their inhibitors with late high-pressure endoleak after endovascular aneurysm repair: long-term results. *J Endovasc Ther*. 2019;26:826–35. https://doi.org/10.1177/1526602819871963

[6] Speelman L, Hellenthal FA, Pulinx B, Bosboom EMH, Breeuwer M, van Sambeek MR, et al. The influence of wall stress on AAA growth and biomarkers. *Eur J Vasc Endovasc Surg*. 2010;39:410–6. https://doi.org/10.1016/j.ejvs.2009.12.021

[7] Wiernicki I, Safranow K, Baranowska-Bosiacka I, Piatek J, Gutowski P. Haptoglobin 2-1 phenotype predicts rapid growth of abdominal aortic aneurysms. *J Vasc Surg*. 2010;52:691–6. https://doi.org/10.1016/j.jvs.2010.03.016

[8] Laronha H, Caldeira J. Structure and function of human matrix metalloproteinases. *Cells*. 2020;9:1076. https://doi.org/10.3390/cells9051076

[9] Singh P, Carraher C, Schwarzbauer JE. Assembly of fibronectin extracellular matrix. *Annu Rev Cell Dev Biol*. 2010;26:397–419. https://doi.org/10.1146/annurev-cellbio-100109-104020

[10] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res*. 2006;69:562–73. https://doi.org/10.1016/j.cardiores.2005.12.002

[11] Burke B. The role of matrix metalloproteinase 7 in innate immunity. *Immunobiology*. 2004;209:51–6. https://doi.org/10.1016/j.imbio.2004.04.005

[12] Baidya SK, Banerjee S, Guti S, Jha T, Adhikari N. Matrix metalloproteinase-8 (MMP-8) and its inhibitors: a minireview. *Eur J Med Chem Rep*. 2024;10:100130. https://doi.org/10.1016/j.ejmcr.2024.100130

[13] Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020;21:9739. https://doi.org/10.3390/ijms21249739

[14] van der Putten RFM, Glatz JFC, Hermens WT. Plasma markers of activated hemostasis in the early diagnosis of acute coronary syndromes. *Clin Chim Acta*. 2006;371:37–54. https://doi.org/10.1016/j.cca.2006.03.005

[15] Kobayashi H, Matsubara S, Yoshimoto C, Shigetomi H, Imanaka S. Tissue factor pathway inhibitors as potential targets for understanding the pathophysiology of preeclampsia. *Biomedicines*. 2023;11:1237. https://doi.org/10.3390/biomedicines11051237

[16] Kurowska P, Mlyczyńska E, Dawid M, Jurek M, Klimczyk D, Dupont J, et al. Review: vaspin (SERPINA12) expression and function in endocrine cells. *Cells*. 2021;10:1710. https://doi.org/10.3390/cells10071710

[17] Turato C, Pontisso P. SERPINB3 (serpin peptidase inhibitor, clade B (ovalbumin), member 3). *Atlas Genet Cytogenet Oncol Haematol*. 2015;19:202–9. https://doi.org/10.4267/2042/56413

[18] Bouton MC, Geiger M, Sheffield WP, Irving JA, Lomas DA, Song S, et al. The under-appreciated world of the serpin family of serine proteinase inhibitors. *EMBO Mol Med*. 2023;15:e17144. https://doi.org/10.15252/emmm.202217144

[19] Syed MH, Zamzam A, Khan H, Singh K, Forbes TL, Rotstein O, et al. Fatty acid binding protein 3 is associated with peripheral arterial disease. *JVS Vasc Sci*. 2020;1:168–75. https://doi.org/10.1016/j.jvssci.2020.08.003

[20] Chaikof EL, Dalman RL, Eskandari MK, Jackson BM, Lee WA, Mansour MA, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg*. 2018;67:2–77.e2. https://doi.org/10.1016/j.jvs.2017.10.044

[21] Feridooni T, Zamzam A, Popkov M, Syed MH, Djahanpour N, Wheatcroft M, et al. Plasma complement component C2: a potential biomarker for predicting abdominal aortic aneurysm related complications. *Sci Rep*. 2022;12:21252. https://doi.org/10.1038/s41598-022-24698-1

[22] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310:2191–4. https://doi.org/10.1001/jama.2013.281053

[23] Torres-Fonseca M, Galan M, Martinez-Lopez D, Cañes L, Roldan-Montero R, Alonso J, et al. Pathophisiology of abdominal aortic aneurysm: biomarkers and novel therapeutic targets. *Clin Investig Arterioscler*. 2019;31:166–77. https://doi.org/10.1016/j.artere.2018.10.003

[24] Vervoort D, Hirode G, Lindsay TF, Tam DY, Kapila V, de Mestral C. One-time screening for abdominal aortic aneurysm in Ontario, Canada: a model-based cost-utility analysis. *CMAJ*. 2024;196:E112–20. https://doi.org/10.1503/cmaj.230913

[25] Piechota-Polanczyk A, Jozkowicz A, Nowak W, Eilenberg W, Neumayer C, Malinski T, et al. The abdominal aortic aneurysm and intraluminal thrombus: current concepts of development and treatment. *Front Cardiovasc Med*. 2015;2:19. https://doi.org/10.3389/fcvm.2015.00019

[26] Skóra J, Dawiskiba T, Zaleska P, Kurcz J, Mastalerz-Migas A, Adamiec R, et al. Prognostic value of tissue factor in patients with abdominal aortic and iliac arterial aneurysms - preliminary study. *Arch Med Sci*. 2013;9:1071–7. https://doi.org/10.5114/aoms

[27] Sagan A, Mrowiecki W, Mikolajczyk TP, Urbanski K, Siedlinski M, Nosalski R, et al. Local inflammation is associated with aortic thrombus formation in abdominal aortic aneurysms. Relationship to clinical risk factors. *Thromb Haemost*. 2012;108:812–23. https://doi.org/10.1160/TH12-05-0339

[28] Wang G, Zeng Y, Chen S, Li D, Li W, Zhou Y, et al. Localization of TFPI-2 in the nucleus modulates MMP-2 gene expression in breast cancer cells. *Sci Rep*. 2017;7:13575. https://doi.org/10.1038/s41598-017-14148-8

SUPPLEMENTARY MATERIAL

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