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Matrix metalloproteinase levels in chronic thoracic aortic dissection

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Abstract

Background—Imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) can lead to aortic wall failure. We hypothesized that patients with aneurysms resulting from chronic descending thoracic aortic dissection have elevated tissue and plasma levels of specific MMPs and decreased tissue levels of TIMPs.

Materials and methods—Aortic tissue was obtained from 25 patients who required surgical repair of descending thoracic aortic aneurysm due to chronic aortic dissection and from 17 organ-donor controls without aortic disease. Tissue levels of MMP-1, -2, -3, -9, -12, and -13 and TIMP-1 and -2 were measured by colorimetric activity assay or enzyme-linked immunosorbent assay and

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confirmed by western blot and immunohistochemistry. Blood obtained from the 25 patients and 15 controls without aortic diseases was used to compare plasma levels of MMP-3, -9, and -12.

Results—Total MMP-1, total MMP-9, and active MMP-9 levels were higher and total MMP-2 levels were lower in dissection tissue than in control tissue. Additionally, the MMP-9/TIMP-1 and active/total MMP-2 ratios were higher and the MMP-2/TIMP-2 ratio was lower in dissection tissue. Furthermore, patients had higher plasma active/total MMP-9 ratios than the controls. Age and hypertension were associated with increased MMP levels.

Conclusions—Increased levels of several MMPs and increased MMP/TIMP ratios in aortic tissue from patients suggest an environment that favors proteolysis, which may promote progressive extracellular matrix destruction and medial degeneration after aortic dissection. An elevated active/total MMP-9 ratio in plasma may be a biomarker for end-stage aneurysm development in patients with chronic thoracic aortic disease.

Keywords

aortic dissection; matrix metalloproteinase; tissue inhibitor of metalloproteinases

1. Introduction

Thoracic aortic dissection (TAD) is a major cause of mortality and morbidity in the United States [1]. A TAD begins as a spontaneous tear through the intima and into the media of the aortic wall. Pulsatile blood entering the tear causes the media to split apart along the length of the vessel. The torn outer aortic wall is weakened and becomes prone to dilatation and rupture, which is usually fatal.

Aortic dissection is caused by the destruction of extracellular matrix (ECM) in the medial layer of the aortic wall. An imbalance in the ratio of matrix metalloproteinases (MMPs) to tissue inhibitors of MMPs (TIMPs) has been shown to play a critical role in ECM destruction in cardiovascular diseases [2]. Numerous studies have also shown that elevated MMP levels may contribute to the development of abdominal aortic aneurysms (AAAs) [3–7]. Elevated MMP levels have also been reported in thoracic aortic aneurysms (TAAs) without dissection [8–14] and in TAD [11, 15–17], but the role of MMPs in aneurysms related to chronic TAD has not been as well studied. Additionally, reports regarding the correlation between aortic MMP levels and plasma MMP levels in AAA have been inconsistent, and the use of plasma MMP levels as a biomarker of aortic disease remains controversial [7, 18, 19].

The objectives of this study were to assess the expression levels of MMPs and TIMPs in patients with aneurysms resulting from chronic descending TAD and to examine these patients' plasma MMP levels. We hypothesized that tissue levels of MMP-1, -2, -3, -9, -12, and -13 are elevated and tissue levels of TIMP-1 and -2 are decreased in aneurysmal descending thoracic aortic tissue from patients with chronic dissection compared with controls. We also hypothesized that patients with chronic descending TAD have elevated plasma MMP levels compared with controls.

2. Materials and methods

2.1. Study design

A case-control design was used for this study. Tissue and plasma levels of MMP-1, -2, -3, -9, -12, and -13 and TIMP-1 and -2 were detected by colorimetric activity assay and enzyme-linked immunosorbent assay (ELISA). Results were confirmed with western blot and immunohistochemistry of tissue samples. We also compared MMP levels in aortic tissue and plasma to determine whether increases in tissue MMP levels were reflected in the plasma. Lastly, we examined the association between the levels of different MMPs and clinical variables.

2.2. Patient enrollment and sample collection

The study group consisted of patients who were referred to our center with the diagnosis of descending thoracic or thoracoabdominal aortic aneurysm due to chronic TAD. Patients with aortitis, ruptured aneurysm, or connective tissue disorders were excluded. Of the 25 patients who met these criteria, 18 patients (72%) had DeBakey type I dissections, and 7 (28%) had DeBakey type III dissections. Aortic samples from TAD patients were obtained from the outer wall of the false lumen at the widest portion of the aneurysm. A preoperative blood sample was also obtained from all TAD patients. Before operation, the TAD patients provided written consent for sample collection, storage, and analysis, as per the study protocol, which was approved by the institutional review boards of Baylor College of Medicine and St. Luke's Episcopal Hospital.

Control samples of descending thoracic aortic tissue were obtained from 17 organ donors who had no history of connective tissue disorders or gross evidence of aortic aneurysm, dissection, coarctation, or previous aortic repair. Control blood samples were collected from 15 blood donors who did not have thoracic aortic aneurysm, aortic dissection, or active cancer. The absence of aneurysms and dissections was confirmed by computed tomography. The choice to use these samples rather than samples from organ donors resulted from concerns regarding the potential effects of trauma, ischemia, and brain death on plasma MMP levels. Demographic and clinical data were collected for patients and control subjects at the time of sample collection (Table 1).

All tissue samples were collected intraoperatively. Periaortic fat and intraluminal thrombus were trimmed away, and the samples were rinsed with saline to remove blood. Each sample was divided into 2 portions; 1 portion was immediately snap frozen in liquid nitrogen and stored at -80°C until batch analysis, and the other portion was placed in buffered 10% formalin and stored at 4°C until batch embedding. Blood samples underwent centrifugation (at 1000 g for 15 min) within 30 minutes of collection. Plasma was stored at -80°C .

2.3. Protein extraction

Samples were lysed in protein lysis buffer, which contained 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM Na_2EDTA , 1 mM EGTA, 2.0 M urea, 1% Brij-35, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 2 mM Na_3VO_4 , and 10 $\mu\text{g/mL}$ of each protease inhibitor (aprotinin, leupeptin, and

pepstatin). For each sample, the total protein concentration was measured in duplicate by using the Bradford method (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA). Samples with concentrations below that required for an assay were further concentrated by centrifugation with a 3000 molecular weight cut-off membrane (Microcon YM-3, Millipore Corporation, Bedford, MA) and re-assayed.

2.4. Colorimetric activity assays

Levels of both active and total MMP-1, -2, and -9 were measured with a commercially available ELISA-like kit (Amersham Biosciences Inc., Piscataway, NJ). Tissue protein concentrations were diluted as indicated for each assay: 2.0 mg/mL for total MMP-1, 10.0 mg/mL for active MMP-1, 0.05 mg/mL for total MMP-2, 4.0 mg/mL for active MMP-2, 1.0 mg/mL for total MMP-9, and 2.0 mg/mL for active MMP-9. Plasma samples were undiluted for the total MMP-1 assay, diluted 1:10 for the total MMP-9 assay, and diluted 1:5 for the active MMP-9 assay. To detect the total MMP activity level of the lysates, MMPs were activated with p-aminophenylmercuric acetate (APMA). Tissue levels of MMPs were indexed to total protein concentration, and plasma levels of MMPs were indexed to total plasma volume.

2.5. Enzyme-linked immunosorbent assays

A sandwich ELISA kit (Amersham Biosciences) was used to measure tissue and plasma levels of MMP-3 and -13 and tissue levels of TIMP-1 and -2. Tissue protein concentrations were diluted as indicated for each assay: 10.0 mg/mL for MMP-3, 2.0 mg/mL for MMP-13, 0.033 mg/mL for TIMP-1, and 3.33 mg/mL for TIMP-2. Plasma samples were undiluted for MMP-3 and MMP-13 assays.

2.6. Fluorescence-quenched substrate cleaving

The enzymatic activity of MMP-12 was analyzed by using a modified technique based on the EnzoLyte 490 kit (AnaSpec, San Jose, CA). This kit is optimized to detect the activity of MMP-12 in biological samples by using a fluorescence-quenched substrate (EDANS/DabcylPlus™ FRET peptide). When this peptide is cleaved into 2 separate fragments by MMPs, its fluorescence is recovered and can be monitored at excitation/emission wavelengths of 340 nm/490 nm. Tissue and plasma samples were incubated with 1 mM APMA for 2 hours to activate MMP-12 and then incubated with substrate at room temperature for 45 minutes. The reaction was stopped with AnaSpec (50 µl) stop solution, after which the fluorescence intensity was measured at 490 nm after excitation at 340 nm. The standard for this assay was MMP-12 purified enzyme (Abcam, Cambridge, MA).

2.7. Western blot

Proteins (40 µg per lane) were separated by 10% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat milk in phosphate-buffered saline with Tween 20 (PBST) for 1 hour at room temperature and then incubated with primary antibody overnight at 4°C. The following primary antibodies were used: mouse monoclonal anti-MMP-1, -2, -3, -9, -12, and -13, mouse monoclonal anti-TIMP-1 and -2 (R&D Systems, Minneapolis, MN), and Glyceraldehyde-3-phosphate

dehydrogenase (GAPDH) (Cell Signaling Technology, Danvers, MA). The membranes were then washed with PBST and incubated with horseradish peroxidase-conjugated anti-mouse secondary antibody at room temperature for 1 hour. Enhanced Chemiluminescence (Amersham Biosciences) was used to visualize bands.

2.8. Representative immunohistochemistry

Immunohistochemistry was performed with a VECTASTAIN® ABC-PEROXIDASE Kit (Vector Labs, Burlingame, CA) according to the manufacturer's instructions. The primary antibodies used for this assay were anti-MMP-1, -2, -3, -9, and -12, anti-TIMP-2 (all from Abcam), anti-MMP-13, and anti-TIMP-1 (both from R&D systems). The tissue sections were developed with a DAB Substrate Kit and then counterstained with VECTOR Hematoxylin QS (both from Vector Labs).

2.9. Statistics

Statistical analysis was performed with SAS 9.3 (SAS Institute Inc. Cary, NC). Interval data are summarized as means with SD, and nominal data are summarized as frequencies with percentages. Baseline characteristics were compared between groups by using the Wilcoxon rank sum test for continuous data and the Fisher exact test for nominal data. Statistical significance was assessed at the 0.05 level. MMP outcome measures were assessed for normality by using quantile-quantile plots. A logarithmic transformation was used to attain approximate normality. Log-transformed MMP measures were compared between groups using independent, two-sample t-tests, and the Holm's stepdown Bonferroni adjustment was used to adjust for multiple comparisons. Figures present means and standard deviations on the original scale. Independent general linear models were used to test for associations between groups and MMP measures while adjusting for clinically important baseline characteristics.

3. Results

3.1. Patient characteristics

The clinical characteristics of the study groups are presented in Table 1. The plasma control subjects were similar in age to the TAD patients, but the control group had a much higher proportion of female members. Control subjects tended to have less hypertension than the TAD patients, and controls were less likely to have a history of smoking or to use cyclooxygenase inhibitors. Controls who donated tissue were significantly younger than TAD patients and were less likely to use anti-lipid medications. Tissue samples from TAD patients were collected 6.2 ± 6.4 years after the onset of dissection. Maximum aortic diameter was significantly larger in TAD patients than in blood-donor controls.

3.2. MMP levels in TAD

Western blots, ELISAs, colorimetric activity assays, and fluorescence-quenched cleaving assays showed that the total protein levels of MMP-1 (Fig. 1A and B) and MMP-9 (Fig. 2A and B) were significantly higher in dissection tissue than in control tissue, whereas the level of total MMP-2 was significantly reduced in dissection tissue (Fig. 3A and B). The active MMP-9 level was also higher in dissection tissue than in control tissue (Fig. 2A), but active

MMP-1 and MMP-2 levels were similar in both groups (Fig. 1A and 3A). Levels of active MMP-1 and total MMP-3 and -13 were higher in dissection patients than in controls; however, these differences were not statistically significant after adjustment for multiple hypothesis tests ($P = 0.08$, 1.0 , and 0.3 , respectively). Consistent with these results, immunostaining of the outer aortic wall of the false lumen showed that MMP-1, -3, -9, -12, and -13 were expressed at higher levels in the media of dissection tissue than in the media of control tissue (Fig. 1C, 4, 2C, 5, and 6, respectively). Immunohistochemistry also showed that MMP-2 was predominantly expressed in the medial layer of the aorta, although this expression was reduced in the dissection tissue samples (Fig. 3C).

Patients had lower plasma levels of total MMP-9 (Fig 2D) than blood-donor controls, but this difference was not statistically significant after adjustment for multiple hypothesis tests ($P = 0.14$). Plasma levels of total MMP-3 and -12 were similar in the TAD patients and blood-donor controls (Fig. 4D and 5 respectively). We could not compare plasma MMP-13 levels between the 2 groups because MMP-13 levels were undetectable in most plasma samples.

3.3. TIMP levels in TAD

Levels of TIMP-1 and -2 in tissue from TAD patients and organ-donor controls were not significantly different (Fig. 7A–C). However, the MMP-9/TIMP-1 ratio, which is an index of net proteolytic activity, was significantly higher in dissection tissue than in control tissue. Conversely, the MMP-2/TIMP-2 ratio was significantly lower (Fig. 7D) in dissection tissue than in control tissue. A summary of the changes in aortic MMP and TIMP levels is presented in Table 2.

3.4. MMP levels in relation to other factors

For aortic tissue MMPs and TIMPs, after adjustment for age, hypertension, history of smoking, COX inhibitor use, and antilipid medication use, there were no longer any significant differences between cases and controls. We found that hypertension was significantly associated with increased ratios of active MMP-9 to total MMP-9 ($P = 0.04$). Increasing age was associated with increased levels of active MMP-2 ($P = 0.007$), decreased levels of total MMP-3 ($P = 0.04$), and increased levels of TIMP-2 ($P = 0.04$). No significant correlations were found between maximum aortic diameter and tissue MMP or TIMP levels in patient samples after adjusting for multiple comparisons.

For plasma MMPs and TIMPs, after adjusting for sex, hypertension, history of smoking, COX inhibitor use, and maximum aortic diameter, we found that patients had significantly lower plasma levels of total MMP-9 ($P = 0.03$) and higher plasma ratios of active to total MMP-9 ($P = 0.002$) than controls. Participants with a history of tobacco use (current or past) tended to have decreased plasma levels of active to total MMP-3 ($P = 0.04$). Female subjects also had lower plasma total MMP-3 levels than male subjects ($P = 0.01$). However, no significant correlations were found between maximum aortic diameter and any of the plasma MMP levels after adjusting for multiple comparisons.

4. Discussion

Normal aortic remodeling is characterized by a balance between the levels of MMPs and TIMPs. This balance provides the appropriate rates of ECM destruction and synthesis necessary to maintain a healthy aortic wall. Disruption of this balance in favor of excessive MMP activity can lead to pathologic aortic wall remodeling and may precipitate aortic wall destruction. AAA and TAA are common degenerative aortic conditions. In these conditions, the proteolytic effects of MMPs on elements of the ECM, primarily elastin and collagen, in the aortic media and adventitia weaken the vessel wall, resulting in vessel dilatation [20]. Studies of mRNA and protein levels have shown increased plasma levels and local expression of MMPs, as well as an imbalance between MMPs and TIMPs, in patients with AAA or TAA [3, 6, 11, 12, 19]. Matrix metalloproteinases-1, -2, -3, -9, -12, and -13 have all been implicated in the onset and progression of AAA and TAA [3, 4, 6, 7, 11, 12, 19, 21]. However, the contribution of MMPs to aneurysm formation in patients with chronic TAD is not well documented.

In this study, we compared the levels of several MMPs and TIMPs in both aortic tissue and plasma from a relatively large number of chronic TAD patients and control subjects. We observed higher levels of total MMP-1 and -9 (but not -3, -12, or -13) and of active MMP-9 in tissue from chronic TAD patients than in control tissue. Our findings are in agreement with prior studies that have examined the role of excessive MMPs in the development of aortic disease [3, 4, 6, 7, 11, 12, 19, 21].

Reports regarding MMP-2 levels have been less consistent, and in our study, although we found that total levels of MMP-2 were decreased in TAD patients, we found that the ratio of active to total MMP-2 was increased. Both increased and decreased total MMP-2 levels have been observed in such patients [15, 17, 21, 22]. Differences in disease stage may account for these inconsistencies. Production of MMP-2 by aortic smooth muscle cells contributes significantly to the overall total MMP-2 levels in the aortic wall (unpublished observation). Severe smooth muscle cell depletion in advanced TAD may result in reduced total MMP-2 levels. In the current study, the mean interval between dissection and operative repair was greater than 6 years. Elevation of the ratio of active/total MMP-2 and a decrease in the ratio of total MMP-2/TIMP-2 in diseased tissue suggest that the net proteolytic activity of MMP-2 is more important than the total amount of enzyme. Nonetheless, it remains unclear in the later stages of the disease whether the changes in MMP-2 levels found in our patients have clinical significance. The temporal variance of MMP-2 levels may also suggest that MMP-2 is involved in both the degradation of aortic tissue and tissue remodeling [15]. Further studies are required to elucidate the significance of MMP-2 levels in different stages of TAD.

Although the levels of MMP-1 and -9 were significantly higher in aortic tissue from TAD patients than in control tissue, after adjustment for covariates, only the plasma level of total MMP-9 was significantly different between TAD patients and blood-donor controls. Whether the balance between active and total MMPs in the plasma plays a crucial role in aortic wall failure remains unknown because, although plasma levels of total MMP-9 were decreased in the TAD patients, the plasma ratio of active/total MMP-9 was significantly

higher in TAD patients. For this reason, a high plasma ratio of active/total MMP-9 may be a biomarker for TAD. Although some AAA data suggest a correlation between MMP levels in plasma and tissue [18], other findings have shown no such correlation [7].

Matrix metalloproteinase activity is partially regulated by TIMPs through the formation of noncovalent complexes. All members of the collagenase, stromelysin, and gelatinase classes of enzymes are inhibited by TIMP-1, whereas TIMP-2 specifically inhibits MMP-2. In our study, TIMP-1 and TIMP-2 levels in the TAD patients and controls were similar, but the MMP-9/TIMP-1 ratio was significantly higher, the MMP-1/TIMP-1 ratio was numerically (but not significantly) higher, and the MMP-2/TIMP-2 ratio was significantly lower in the TAD patients. This relative imbalance, which favors net proteolysis, may have important implications for progressive matrix destruction and aneurysm formation.

It is well established that MMPs play a central role in the growth and rupture of aortic aneurysms [23, 24]. However, findings regarding the relationship between MMP levels and aortic expansion have been inconclusive. Although several studies have shown that levels of MMP-2 and MMP-9 positively correlate with aneurysm diameter [5, 25], others reports have shown a negative association [4, 19]. In the present study, aortic diameter was not associated with any of the MMP or TIMP levels in tissue or plasma (after adjusting for multiple comparisons), which is consistent with a previous report [26]. It should be noted that our study participants were patients with end-stage chronic TAD. Aortic aneurysm is a multi-stage paradigm, and MMP-mediated aortic elastolysis may occur early during aneurysm formation. Additionally, because the tissue samples from the TAD patients in our study were obtained from the outer wall of the false lumen, they may not reflect the pathological process occurring in aneurysms of the aortic wall that are not caused by dissection.

Although our study extends the current knowledge of descending thoracic aortic dissection, certain limitations are worth noting. Some differences in the characteristics of the patient and control groups were unavoidable because of the limitations in sample availability. The difference in age, history of hypertension, history of smoking, COX inhibitor use, and antilipid medication use between the TAD patients and the organ-donor control subjects (for tissue analysis) and the gender differences between the TAD patients and blood-donor control subjects (for plasma analysis) may have contributed to the differences observed in MMP levels. In our study, hypertension was significantly associated with increased ratios of active/total MMP-9, and age was associated with increased active MMP-2 and TIMP-2 levels but not with a net change in the ratio of MMP-2/TIMP-2, suggesting that even though there was a change in total levels, age was not a factor in net proteolysis. However, after adjustment for age, hypertension, history of smoking, COX inhibitor use, and antilipid medication use, there were no longer any significant differences between cases and tissue controls. Nonetheless, using aortic tissue from organ donors precluded the possibility of matching TAD patients and tissue donors completely. Validating the correlation between these clinical characteristics and total MMP levels may require additional studies with larger groups of patients.

Furthermore, all TAD patients in this study required surgical intervention for aortic expansion or the development of symptoms, so the MMP and TIMP profiles observed may

reflect late-stage disease; it is unclear whether similar profiles are present in earlier stages of post-dissection aneurysm formation. Finally, we did not examine mRNA levels of MMPs, so our results reflect protein translation rather than protein transcription. Because MMPs are regulated at the level of transcription, determining whether MMP transcription is elevated will increase the understanding of what is occurring on a cellular level. However, MMP activity and the imbalance between proteases and their inhibitors are more clinically relevant. Additional studies in animal models and human subjects should be conducted to determine the causal relationships among MMP dysregulation, TAD, post-dissection aneurysm formation, and aortic rupture. In addition to providing insights into the mechanisms driving these disease processes, such studies will help establish whether MMP inhibition should be explored as a potential therapeutic strategy for these patients.

In summary, the aortic tissue from patients with descending thoracic aortic aneurysm due to chronic dissection had increased levels of several MMPs, as well as an increased MMP-9/TIMP-1 ratio. These conditions favor proteolysis. We also found that active/total MMP-9 ratios were increased in plasma and may potentially be used as a biomarker for patients with end-stage aneurysmal chronic TAD. Overexpression of MMPs may play a role in progressive ECM degradation and medial degeneration.

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Fig. 1. Increased MMP-1 levels in chronic TAD tissue versus control tissue as observed by the use of (A) colorimetric activity assay (total and active), (B) western blot, and (C) immunohistochemical staining.

Fig. 2.

Increased MMP-9 levels in chronic TAD tissue versus control tissue as observed by the use of (A) colorimetric activity assay (total and active), (B) western blot, and (C) immunohistochemical staining. (D) Plasma total (but not active) MMP-9 levels were decreased in chronic TAD patients versus control patients. Results are displayed as mean + SD. Adv = adventitia; FL = false lumen; Int = intima.

Fig. 3. Decreased MMP-2 levels in chronic TAD tissue versus control tissue as observed by the use of (A) colorimetric activity assay (total), (B) western blot, and (C) immunohistochemical staining. Results are displayed as mean + SD. Adv = adventitia; FL = false lumen; Int = intima.

Fig. 4. MMP-3 levels in chronic TAD tissue versus control tissue as observed by the use of (A) ELISA, (B) Western blot, and (C) immunohistochemical staining. (D) In chronic TAD patients versus control patients, plasma MMP-3 levels were not elevated in univariate analysis. Results are displayed as mean + SD. Adv = adventitia; FL = false lumen; Int = intima.

Fig. 5. MMP-12 levels in chronic TAD tissue versus control tissue as observed by the use of (A) fluorescence-quenched cleaving assay, (B) western blot, and (C) immunohistochemical staining. (D) Plasma MMP-12 levels were not elevated in chronic TAD patients versus control patients. Results are displayed as mean + SD. Adv = adventitia; FL = false lumen; In = intima.

Fig. 6. Increased MMP-13 levels in chronic TAD tissue versus control tissue as observed by the use of (A) ELISA, (B) western blot, and (C) immunohistochemical staining. Results are displayed as mean + SD. Adv = adventitia; FL = false lumen; Int = intima.

Fig. 7.

Levels of TIMP-1 did not differ between groups, whereas TIMP-2 levels showed varying results (according to the test used) in chronic TAD tissue versus control tissue as observed by the use of (A) ELISA, (B) western blot, and (C) immunohistochemical staining. (D) MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios were significantly altered in chronic TAD patients. Results are displayed as mean + SD. Adv = adventitia; FL = false lumen; Int = intima.

Table 1

Clinical characteristics of the study groups.

Variables	Dissection		Control Aorta		Control Blood	
	n = 25	n = 17	n = 17	P value [*]	n = 15	P value [*]
Age (y)	60.8 ± 9.7	34.2 ± 14.7		<0.001	60.6 ± 16.1	0.96
Female	5 (20%)	4 (24%)		1.00	9 (60%)	0.02
Hypertension	24 (96%)	1 (6%)		<0.001	9 (60%)	0.007
COPD	6 (24%)	0		0.07	2 (13%)	0.69
Diabetes mellitus	2 (8%)	0		0.51	1 (7%)	1.0
Stroke	2 (8%)	0		0.51	0	0.52
CAD	5 (20%)	0		0.07	0	0.14
Smoking history	19 (76%)	5/13 [†] (39%)		0.04	4 (27%)	0.003
Medication use						
COX inhibitor	7 (28%)	0		0.03	10 (67%)	0.02
Antilipid	12 (48%)	1/14 [†] (7%)		0.01	5 (33%)	0.51
Max. aortic diameter (cm)	6.7±1.1	N/A		N/A	3.2±0.4	<0.001

CAD = coronary artery disease; COPD = chronic obstructive pulmonary disease; COX = cyclooxygenase; Max = maximum; N/A = Data not available.

^{*} Control group vs dissection group. *P*-values were generated by the Wilcoxon rank sum test for continuous variables and the Fisher exact test for nominal variables.[†] These data were not available for all patients in the control aorta group.

Table 2

Summary of the changes in aortic MMP and TIMP levels in patients with aneurysmal chronic TAD compared with controls.

Level Increased	Level Decreased	Level Similar to Controls
MMP-1 (total)	MMP2 (total)	Active/total MMP-9
MMP-9 (total)	Total MMP-2/TIMP-2	MMP-1 (active)
MMP-9 (active)	Plasma MMP-9 (total)*	Active/total MMP-1
Active/total MMP-2		MMP-2 (active)
MMP-9/TIMP-1		MMP-3 (total)
Plasma MMP-3 (active)		MMP-3 (active)
Plasma active/total MMP-9*		Active/total MMP-3
		MMP-12 (total)
		MMP-13 (total)
		TIMP-1
		TIMP-2
		MMP1/TIMP1
		Plasma MMP-9 (active)
		Plasma MMP-3 (total)
		Plasma active/total MMP-3
		Plasma MMP-12

* Per multivariable analysis.