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Plasma levels of cathepsins L, K, and V and risks of abdominal aortic aneurysms: a randomized population-based study

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Abstract

Background—Cathepsin L (CatL), cathepsin K (CatK), and cathepsin V (CatV) are potent elastases implicated in human arterial wall remodeling. Whether plasma levels of these cathepsins are altered in patients with abdominal aortic aneurysms (AAAs) remains unknown.

Methods and Results—Plasma samples were collected from 476 male AAA patients and 200 age-matched male controls to determine CatL, CatK, and CatV levels by ELISA. Student's *t*-test demonstrated significantly higher plasma CatL levels in AAA patients than in controls ($P < 0.0001$), whereas CatK and CatV levels were lower in AAA patients than in controls ($P = 0.052$, $P = 0.025$). ROC curve analysis confirmed higher plasma CatL levels in AAA patients than in controls ($P < 0.001$). As potential confounders, current smoking and use of angiotensin-converting enzyme (ACE) inhibitors, aspirin, clopidogrel, and statins associated with significantly increased plasma CatL. Pearson's correlation test demonstrated that plasma CatL associated positively with CatS ($r = 0.43$, $P < 0.0001$), body-mass index (BMI) ($r = 0.07$, $P = 0.047$) and maximal aortic diameter ($r = 0.29$, $P < 0.001$), and negatively with lowest measured ankle-brachial index (ABI) ($r = -0.22$, $P < 0.001$). Plasma CatL remained associated positively with CatS ($r = 0.43$, $P < 0.0001$) and aortic diameter ($r = 0.212$, $P < 0.001$) and negatively with ABI ($r = -0.10$, $P = 0.011$) after adjusting for the aforementioned potential confounders in a partial correlation analysis. Multivariate logistic regression analysis indicated that plasma CatL was a risk factor of AAA before (odds ratio [OR] = 3.04, $P < 0.001$) and after (OR = 2.42, $P < 0.001$) the same confounder adjustment.

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Author Contributions

BJL and JW performed the ELISA analysis. JSL participated in patient sample collection, statistical analysis, and manuscript writing. XC participated in initial experimental design. GPS designed the study, analyzed the data, and wrote the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Conclusions—Correlation of plasma CatL levels with aortic diameter and the lowest ABI suggest that this cysteinyl protease plays a detrimental role in the pathogenesis of human peripheral arterial diseases and AAAs.

Keywords

cathepsin L; cathepsin K; cathepsin V; abdominal aortic aneurysm; aortic diameter; ankle–brachial index

1. Introduction

The pathogenesis of arterial diseases, including atherosclerosis and abdominal aortic aneurysms (AAAs), involves substantial degradation of the arterial wall matrix proteins, including elastin, collagen, laminin, fibronectin, and many others — a process mediated by proteolytic enzymes. Several families of proteases, including matrix metalloproteinases (MMPs), cysteine protease cathepsins, and serine proteases, play essential roles in the pathogenesis of atherosclerosis and AAAs in humans and animals. For example, many of the cysteinyl cathepsins — such as cathepsin S (CatS), cathepsin K (CatK), cathepsin L (CatL), and cathepsin V (CatV) — are potent elastases and collagenases [1, 2], expressed highly in human arterial lesions of atherosclerosis and AAAs [3–6]. Among all cathepsins, CatV is the most potent mammalian elastase [2, 6], but its direct involvement in atherosclerosis or AAAs remains untested. But in mice, the absence of CatS [7], CatK [8], and CatL [9] protected against diet-induced atherosclerosis. These cathepsins also participate directly in experimental AAAs. Using both angiotensin-II (Ang-II) infusion-induced and aortic elastase perfusion-induced AAAs in mice, we demonstrated that deficiency of CatS [10], CatK [11], and CatL [12] protected against AAA formation.

We recently showed that plasma CatS was elevated in AAA patients, and served as an independent AAA risk factor (odds ratio [OR] = 1.332, $P < 0.001$) that associated positively with AAA size ($r = 0.291$, $P < 0.001$) and negatively with the lowest ankle–brachial index (ABI) ($r = -0.225$, $P < 0.001$). These associations persisted after adjustment for common AAA risk factors ($r = 0.256$, $P < 0.001$; $r = -0.124$, $P = 0.002$, respectively) [13]. This current study examined whether plasma levels of other potent elastinolytic cathepsins, including CatL, CatK, and CatV, also associated with AAA risk and aortic size.

2. Materials and methods

2.1. Study population

In an ongoing randomized population–based screening trial for AAAs, peripheral arterial disease (PAD), and hypertension in more than 50,000 men 65–74 years of age in the mid-region of Denmark [14], baseline plasma samples were taken consecutively at diagnosis of 476 AAA patients and 200 age-matched controls without AAA or PAD. AAA was defined as having maximal aortic diameter greater than 30 mm, and PAD was defined as an ABI lower than 0.90 or >1.4 . AAA cases among first-degree relatives, smoking status, coexisting diabetes mellitus, hypertension, and use of β -blockers, angiotensin-converting enzyme (ACE) inhibitors, and statins were recorded. Body-mass index (BMI) and systolic and diastolic blood pressure were also measured and recorded. Plasma CatS and ankle systolic blood pressure was measured as previously validated and reported [13, 15], and maximal anterior–posterior diameter of the infrarenal aorta was measured in the peak of the systole from inner edge to inner edge of the aorta. The lowest ABI was calculated by dividing the lowest recorded ankle blood pressure by the brachial systolic blood pressure. Patients with AAAs less than 50 mm were offered annual control scans by the screening team; patients with AAAs measuring 50 mm or larger were referred for a computed tomography (CT) scan

and vascular surgical evaluation. The interobserver variation of aortic diameter measurements was 1.52 mm [16]. Growth rates of small AAAs in patients kept under surveillance were calculated by individual linear regression analysis, using all observations. Blood samples were centrifuged at 3000 g for 12 minutes, aliquoted, and stored at -80°C until analysis was performed. All subjects gave informed consent before participating, and the local Ethics Committee of the Viborg Hospital, Denmark, approved the study, which was performed in accordance with the Helsinki Declaration. The Partners Human Research Committee (Boston, MA, USA) also approved the use of non-coded human samples.

2.2. ELISA

Plasma total CatL and CatV levels were determined blindly using ELISA DueSet kits from R&D Systems (Minneapolis, MN; catalog numbers DY952 and DY1080), and plasma total CatK levels were determined using ELISA kits from BioTang Inc. (Waltham, MA), according to the manufacturer's instructions.

2.3. Statistics

Dichotomous variables were expressed as proportions and compared by the chi-square test, and reported as odds ratios. One sample Kolmogorov–Smirnov test and probability plot (not shown) were used to determine whether continuous variables were normally distributed, and compared between controls and cases with Student's *t*-test. Receiver–operator characteristic (ROC) curve analyses were performed non-parametrically to test the predictive value of the tests, concerning the prediction of AAA cases. For analyses of the ROC curves, the null hypothesis was that the test performed similarly to the diagonal line — i.e., the area under the curve was 0.5. If the lowest 95% confidence limit for the area under the curve was above 0.5, a significant predictive test was present. The optimal cut-off points were determined, and the respective sensitivity and specificity were calculated. The potential markers were then tested as independent predictors of AAA by logistic regression analysis, adjusting for smoking, hypertension, β -blocker use, low-dose aspirin use, statin use, systolic blood pressure (SBP), age, BMI, and lowest ABI. The associations between the potential serological biomarkers were then correlated to maximal aortic diameter, lowest ABI, and AAA growth rate by Pearson's correlation analysis. The best potential serological biomarker was then tested for independent association with maximal aortic diameter and with lowest ABI, respectively, with Pearson's partial correlation analysis, adjusting for the aforementioned potential confounders.

3. RESULTS

3.1. Increased plasma CatL levels in AAA patients

Of more than 50,000 volunteers, more than 25,000 were randomized for screening for PAD, AAA, and hypertension. Approximately 75% of those randomized attended the screening [14]. Of the first 476 consecutively diagnosed cases of AAAs (3.3%), 385 had small AAAs (aortic diameters smaller than 50 mm) and were offered surveillance ranging from 0.52 to 3.1 years, with an average of 1.69 ± 0.57 (mean $\pm$ SD) years. Patients with AAAs measuring 50 mm or more were referred for a CT scan and to the vascular surgical department to be evaluated for potential repair. Patients not given a surgical referral were followed by the screening team. Demographic factors and potential confounders, as well as aortic diameters, lowest ABI, growth rates, and serological findings have been reported previously [13]. The mean ages were 70.0 ± 2.8 (mean $\pm$ SD) years and 69.6 ± 2.8 (mean $\pm$ SD) years among those without and with AAAs, respectively.

In this study, we performed Student's *t*-test and found that 476 AAA patients had significantly higher plasma CatL levels than 200 age-matched controls (1.38 ± 0.71 vs. 0.99

± 0.70 ng/mL, $P < 0.0001$). In contrast, plasma CatK levels (105.96 ± 50.89 vs. 116.38 ± 59.78 pmol/mL, $P = 0.052$) were not significantly different between AAA patients and controls. Plasma CatV levels were even significantly reduced in AAA patients compared with controls (192.26 ± 351.20 vs. 278.36 ± 484.00 pg/mL, $P = 0.025$) (Figure 1). We examined the difference of familiar disposition, current smoking, diabetes mellitus, hypertension, ACE inhibitor use, β -blocker use, low-dose aspirin or clopidogrel use, statin use, SBP, diastolic blood pressure (DBP), age, plasma CatS levels, BMI, lowest ABI, and maximal aortic diameters between patients with AAAs and age-matched controls. Among all tested dichotomous variables, there was significantly more current smoking ($P < 0.0001$), hypertension ($P = 0.042$), β -blocker use ($P = 0.048$), low-dose aspirin or clopidogrel use ($P < 0.0001$), and statin use ($P < 0.0001$) among AAA patients than among non-AAA controls (Table 1). Current smoking (1.34 ± 0.68 vs. 1.22 ± 0.74 ng/mL, $P = 0.010$), ACE inhibitor use (1.34 ± 0.81 vs. 1.23 ± 0.69 ng/mL, $P = 0.036$), low-dose aspirin or clopidogrel use (1.37 ± 0.72 vs. 1.20 ± 0.72 ng/mL, $P < 0.001$), and statin use (1.33 ± 0.74 vs. 1.19 ± 0.71 ng/mL, $P = 0.006$) increased plasma CatL levels significantly (Table 1). In contrast, statin use decreased plasma CatK levels (112.3 ± 54.5 vs. 104.8 ± 51.9 pmol/mL, $P = 0.022$), and current smoking decreased plasma CatV levels (247.1 ± 438 vs. 177.7 ± 360 pg/mL, $P = 0.004$) (Table 1). Among all continuous variables, AAA patients had significantly higher SBP ($P = 0.008$), DBP ($P < 0.0001$), plasma CatS ($P < 0.0001$), BMI ($P < 0.0001$), and maximal aortic diameter ($P < 0.0001$), but significantly lower lowest ABI ($P < 0.0001$) than those in the control subjects (Table 1). Pearson's correlation test demonstrated that plasma CatL correlated negatively with lowest ABI ($r = -0.22$, $P < 0.001$), positively with plasma CatS ($r = 0.43$, $P < 0.0001$), maximal aortic diameters ($r = 0.29$, $P < 0.001$), and weakly but significantly with BMI ($r = 0.07$, $P = 0.047$) (Table 1). In contrast, plasma CatK only weakly and negatively correlated with SBP ($r = -0.07$, $P = 0.049$). Plasma CatV levels correlated positively with CatS ($r = 0.13$, $P < 0.01$) and lowest ABI ($r = 0.13$, $P < 0.01$) and negatively with maximal aortic diameters ($r = -0.11$, $P = 0.038$). Plasma CatL, CatK, and CatV levels did not correlate with AAA growth rate (Table 1). Our observations suggest that plasma CatL level is a risk factor for human AAA and peripheral arterial occlusive disease, whereas plasma CatV level may be protective against arterial diseases (Table 1).

3.2. Plasma CatL is an independent risk factor of human AAA

ROC curve analysis demonstrated the significant differences in human plasma CatL levels between AAA patients and controls (AUC [area under the ROC curve] = 0.703, $P < 0.001$), with optimal sensitivity and specificity of 0.65 and 0.65. In contrast, both plasma CatK and CatV were not risk factors, but rather protective factors against human AAA (AUC = 0.449, $P = 0.039$ and AUC = 0.432, $P = 0.005$), with optimal sensitivities and specificities of 0.55 and 0.55 vs. 0.55 and 0.55 (Figure 2). Univariate logistic regression analysis showed that plasma CatL was a significant and strong risk factor of AAA (odds ratio [OR]: 3.04 [2.14–4.33, 95% C.I.], $P < 0.001$), whereas both plasma CatK (OR: 0.71 [0.52–0.96, 95% C.I.], $P = 0.028$) and CatV (OR: 0.95 [0.92–0.99, 95% C.I.], $P = 0.017$) seemed protective against AAA (Table 2). A multiple logistic regression analysis demonstrated that plasma CatL remained as a significant and strong risk factor for human AAA (OR: 2.42 [1.66–3.52, 95% C.I.], $P < 0.001$) after adjustment for smoking, hypertension, β -blocker use, low-dose aspirin use, statin use, SBP, age, BMI, and lowest ABI. After the same adjustment, however, CatK (OR: 0.78 [0.54–1.15, 95% C.I.], $P = 0.196$) and CatV (OR: 0.99 [0.94–1.04, 95% C.I.], $P = 0.761$) were not associated with the risk of AAA in this population (Table 2).

3.3. Plasma CatL levels correlate with AAA aortic diameter and lowest ABI

Pearson's univariate and partial correlation analyses demonstrated that plasma CatL correlated positively and strongly with AAA maximal aortic diameter ($r = 0.29$, $P < 0.001$ vs. $r = 0.232$, $P < 0.001$) before and after adjustment for smoking, hypertension, β -blocker

use, low-dose aspirin use, statin use, SBP, age, BMI, and lowest ABI. Plasma CatK did not correlate with aortic diameter ($r = -0.05$, $P = 0.248$ vs. $r = -0.019$, $P = 0.633$) in either the univariate or partial correlation analyses. In contrast, plasma CatV levels correlated negatively with maximal aortic diameter ($r = -0.11$, $P = 0.003$ vs. $r = -0.093$, $P = 0.021$) before and after the same adjustments (Table 3).

When lowest ABI, representing peripheral arterial occlusive disease, was considered as the variable, we demonstrated that only plasma CatL ($r = -0.22$, $P < 0.001$ vs. $r = -0.10$, $P = 0.011$), not CatK ($r = -0.06$, $P = 0.11$ vs. $r = -0.01$, $P = 0.983$) or CatV ($r = -0.01$, $P = 0.986$ vs. $r = 0.03$, $P = 0.508$), correlated negatively with lowest ABI before and after the same adjustment of potential AAA risk factors (Table 3).

4. Discussion

Cathepsins S, K, L, and V are potent elastases and collagenases [1, 2]. Increased expression of these proteases in human atherosclerotic and AAA lesions [3-6] suggests their involvement in the pathological events of these arterial diseases, a hypothesis that has been tested in experimental atherosclerosis [7-9] and experimental AAAs [10-12]. Although whether these cysteinyl cathepsins participate directly in human AAA is unknown, increased expression of these proteases in human AAA lesions [4, 5] may lead to concurrent increases in the circulation. We therefore may measure their levels in plasma as biomarkers to predict indirectly AAA risk in humans. Plasma total and mature CatS levels were higher in male AAA patients than in age-matched male controls, and both correlated with AAA diameters ($r = 0.105$, $P = 0.006$ and $r = 0.268$, $P < 0.001$) in a Pearson's correlation test [13]. After adjustment for common AAA risk factors (familial AAA, smoking, diabetes, hypertension, ACE inhibitor use, β -blocker use, statin use, SBP, DBP, peripheral arterial disease, BMI, and cystatin C), plasma total CatS ($r = 0.277$, $P < 0.001$) and mature CatS (not shown) remained associated significantly with AAA diameter [13]. These observations suggest that plasma CatS level is a risk factor of human AAAs. This hypothesis was tested in both a ROC curve analysis and a logistic regression analysis [13]. In a recent independent study, Koole et al. [17] extracted proteins from human aortic lesion biopsies from 329 patients with AAAs and measured biopsy CatB and CatS levels. Spearman's correlation test revealed that lesion biopsy CatB ($r = 0.384$, $P < 0.001$ vs. $P < 0.001$) and CatS ($r = 0.467$, $P < 0.001$ vs. $P < 0.001$) levels associated significantly with a secreted form of the tumor necrosis factor (TNF) receptor glycoprotein osteoprotegerin, before and after adjustment for aneurysm diameter, age, sex, hypertension, smoking, diabetes, history of myocardial infarction, and chronic obstructive pulmonary disease. This glycoprotein also associated significantly with AAA diameter ($r = 0.196$, $P = 0.001$ vs. $P = 0.001$) before and after the same adjustment [17]. Similar observations were obtained from 259 patients with asymptomatic AAAs. Lesion CatB ($r = 0.383$, $P < 0.001$ vs. $P < 0.001$) and CatS ($r = 0.473$, $P < 0.001$ vs. $P < 0.001$) levels associated with osteoprotegerin before and after the same adjustment [17]. These results suggest that lesion CatB and CatS levels associate indirectly with AAA size, although such a hypothesis was not tested directly in the study by Koole et al.

The current study showed increased plasma CatL in 476 male AAA patients compared with 200 age-matched male controls. Plasma CatL associated positively and significantly with maximal aortic diameter, but negatively and significantly with lowest ABI before and after adjustment (Tables 1 and 3). However, Pearson's correlation test did not show a significant correlation between plasma CatL and aneurysmal annual growth rate ($r = 0.10$, $P = 0.069$) (Table 1). This observation is similar to what we have reported in plasma CatS levels, which also showed no association with AAA growth rate. Such insignificant correlations may be due to the relatively short follow-up time of this population of patients (1.69 ± 0.57 years, mean $\pm$ SD) [13], although this hypothesis can be tested after few more years of annual

surveillance. Nevertheless, significant associations between plasma CatL with maximal aortic size and with lowest ABI before and after adjustment of common AAA risk factors suggest that CatL contributes to the pathogenesis of both AAA and peripheral arterial occlusive disease, thereby serving as a risk factor for these human arterial diseases. Indeed, univariate and multiple logistic regression demonstrated that plasma CatL is an independent risk factor of AAA with an OR of 3.04 (2.14, 4.33) and 2.42 (1.66; 3.52), before and after adjustment for other common AAA risk factors (Table 2).

Like CatS [4] and CatL [5], CatK is a potent elastase and collagenase that is highly expressed in human AAA lesions [4]. High expression of CatV was detected in human atheroma lesions and stenotic valves [2, 6]. Although no reports exist regarding CatV expression in human AAA, human AAA lesions may contain more CatV proteins than normal aortas. In these lesions, macrophages are the major cell type expressing cathepsins B, S, L, and K [18]. While monocytes did not express CatV, IL-3-induced differentiation of monocytes into macrophages increased CatV expression [2]. Macrophages in human AAA lesions therefore should express CatV, but we detected even lower plasma CatK levels in AAA patients than in controls — although these did not reach statistical significance (105.96 ± 50.89 vs. 116.38 ± 59.78 pmol/mL, $P = 0.052$). Further, plasma CatV levels also were significantly lower in AAA patients than in controls (192.26 ± 351.20 vs. 278.36 ± 484.00 pg/mL, $P = 0.025$). We currently do not have an explanation for these observations. The absence of cystatin C in human AAA lesions [4] magnified already increased CatK [4] and possibly CatV activities in situ. Increased levels of CatK and CatV in human AAA lesions therefore may be sufficient to damage the arterial wall. This hypothesis is supported by the observation that CatK deficiency protected mice from elastase perfusion-induced AAAs in mice [11]. Reduced plasma CatK and CatV in AAA patients from this study may not suggest their indication as biomarkers or risk factors for human AAA, but this does not disprove their role in AAA pathogenesis.

Using atherosclerosis-prone low-density lipoprotein receptor (LDLr)-deficient mice consuming an atherogenic diet, aortic elastase perfusion-induced AAA and chronic Ang-II infusion-induced AAA in mice, we demonstrated a direct participation of both CatL and CatS in atherosclerosis [7, 9] and AAA [10, 12]. Significant associations between AAA sizes and lowest ABI with both CatS from our previous study [13] and CatL from this study supported further an involvement of both CatS and CatL in human AAA and atherosclerosis. AAA and atherosclerosis are two different arterial diseases with different pathologies and even different pathogenic mechanisms. However, atherosclerosis is an important risk factor of AAA [19] and patients with AAA frequently have atherosclerosis [20]. Both these arterial diseases share many common pathologic events, including medial elastic catabolism, inflammatory cell infiltration, angiogenesis, vascular cell migration, proliferation, and apoptosis. Both CatS and CatL are potent elastases [1], contribute to blood-borne leukocyte transendothelium migration [7, 9], and affect directly arterial wall cell proliferation and angiogenesis [10, 12]. These molecular mechanisms of CatS and CatL support the hypothesis from this study and our previous observation [13] that both these cathepsins may serve as important risk factors of human AAAs and peripheral arterial diseases.

Of note, the sources of these cathepsins in the circulation remained untested. AAA is an arterial chronic inflammatory disease. Prior studies showed that plasma C-reactive protein (CRP) levels are significantly higher in AAA patients than those from controls ($P < 0.0001$) and correlate significantly with AAA sizes ($P < 0.0001$) [21]. Inflammatory cells in the aortic wall and within the circulation may be one of the major sources. Aortic wall from AAA lesions contain large quantity of inflammatory cytokines IL6 and TNF- α and chemokine IL8 [22]. These cytokines are also well-known stimuli of cathepsin productions from vascular smooth muscle cells and endothelial cells [23, 24]. We currently do not have a

complete view of any inflammatory marker for this new population, such as CRP or inflammatory cytokines. Such limitation prevented us from direct assessment of inflammation-associated plasma cathepsin production. However, within the same population, Pearson's correlation test demonstrated a significant correlation between CatL and CatS ($r = 0.43$, $P < 0.0001$) (Table 1), suggesting that both cathepsins come from similar sources. Detailed assessment of plasma leukocytes counts, inflammatory cytokine levels, and other inflammatory biomarkers such as CRP or serum amyloid A may help to establish a connection between inflammation and plasma cathepsins.

Together, although all three tested cathepsins (K, L, and V) are highly expressed in human AAA and atherosclerotic lesions [2-6], only plasma CatL — not CatK or CatV — associated positively with maximal aortic diameter and negatively with lowest ABI in AAA patients, predicting the risk of developing AAAs and peripheral arterial occlusive disease. Targeting this protease may have therapeutic potential among AAA patients. However, insignificant association between plasma CatL and AAA growth rate (Table 1) from this short follow-up study and significant but weak sensitivity and specificity from the ROC curve analysis to discriminate between AAA patients and controls (Figure 2) suggest that it is premature to use plasma CatL as a human AAA biomarker. Large population and much longer follow-up may be required in the future to test this possibility.

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Abbreviations

AAA	abdominal aortic aneurysm
CatL	cathepsin L
CatK	cathepsin K
CatV	cathepsin V
ABI	ankle-brachial index
ECM	extracellular matrix
MMP	metalloproteinase
PAD	peripheral arterial disease
ACE	angiotensin-converting enzyme
BMI	body-mass index
CT	computed tomography
ROC	receiver-operator characteristic
OR	odds ratio
AUC	area under the ROC curve

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Highlights

- We determined plasma cathepsin L, cathepsin K, and cathepsin V levels in 476 male patients with abdominal aortic aneurysm (AAAs) and 200 age-matched male controls.
- We demonstrated significant higher plasma cathepsin L levels in patients with AAA than in controls.
- We demonstrated that plasma cathepsin L correlated with maximal aortic diameter and measured ankle–brachial index in this population.
- We demonstrated that plasma cathepsin L was a significant risk factor of human AAAs and peripheral arterial occlusive disease.

Figure 1.

Plasma CatL, CatK, and CatV levels between patients with AAA (n=476) and age-matched non-AAA controls (n=200). Data are mean $\pm$ SEM, $P < 0.05$ is considered statistically significant, Student *t* test.

Variables	Area under the curve	Standard error	P-value	95% C.I.	Sensitivity	Specificity
CatL	0.703	0.022	<0.001	0.660 – 0.746	0.65	0.65
CatK	0.449	0.025	0.039	0.401 – 0.498	0.55	0.55
CatV	0.432	0.025	0.005	0.383 – 0.480	0.55	0.55

Figure 2.

ROC curve analysis of plasma CatL, CatK, and CatV levels between patients with AAA and age-matched non-AAA controls.

Table 1

Dichotomous and continuous variables between AAA patients and controls and their associations with plasma CatL, CatK, and CatV levels.

Dichotomy variables	Control vs. AAA	CatL (ng/mL) (No vs. Yes)	CatK (pmol/L) (No vs. Yes)	CatV (pg/mL) (No vs. Yes)
Familiar disposition	0.03 0.07	1.26 (0.72) 1.33 (0.76)	108.4 (53.7) 110.9 (51.6)	422.0 (16.9) 280.6 (46.1)
Current smoking	0.17 0.42 ^{**}	1.22 (0.74) 1.34 (0.68) ^{**}	109.5 (51.5) 106.9 (56.8)	247.1 (438) 177.7 (360) ^{**}
Diabetes mellitus	0.15 0.10	1.25 (0.69) 1.35 (0.91)	109.1 (53.1) 105.0 (55.9)	227.1 (415) 194.4 (409)
Hypertension	0.42 0.52 [*]	1.26(0.72) 1.27(0.74)	108.0 (52.4) 110.0 (54.8)	225.6 (413) 219.6 (417)
ACE inhibitor use	0.21 0.26	1.23 (0.69) 1.34 (0.81) [*]	110.4 (51.6) 104.3 (59.0)	232.7 (429) 201.3 (379)
β-blocker use	0.22 0.29 [*]	1.26 (0.74) 1.31 (0.68)	109.9 (56.1) 105.7 (46.1)	226.3 (398) 222.0 (467)
Low-dose aspirin or clopidogrel use	0.24 0.47 ^{**}	1.20 (0.72) 1.37 (0.72) ^{**}	111.1 (57.3) 105.3 (46.8)	212.4 (381) 241.8 (463)
Statin use	0.35 0.52 ^{**}	1.19 (0.71) 1.33 (0.74) ^{**}	112.3 (54.5) 104.8 (51.9) [*]	240.6 (434) 206.3 (395)

Continuous variables	Mean (SD)	CatL (Pearson's r)	CatK (Pearson's r)	CatV (Pearson's r)
Systolic blood pressure (mm Hg)	148 (19.4) 155 (21.4) ^{**}	-0.019	-0.07 [*]	-0.02
Diastolic blood pressure (mm Hg)	81.0 (10.5) 88.1 (12.2) ^{**}	0.03	<0.01	<0.01
Age (years)	69.6 (2.8) 70.0 (2.8)	0.03	-0.02	-0.09 ^{**}
Cathepsin S (ng/mL)	10.8 (3.8) 14.7 (4.5) ^{**}	0.43 ^{**}	0.05	0.13 ^{**}
Body-mass index	26.2 (3.3) 27.2 (3.5) ^{**}	0.07 [*]	-0.04	0.03
Lowest ABI (mm Hg)	1.10 (0.12) 0.94 (0.20) ^{**}	-0.22 ^{**}	0.02	0.13 ^{**}
Max. aortic diameter (mm)	18.7 (4.6) 40.5 (11.9) ^{**}	0.29 ^{**}	-0.05	-0.11 [*]
AAA growth rate (mm/year)	2.71 (2.58)	0.10	0.01	-0.05

* $P < 0.05$

** $P < 0.01$.

Table 2

Univariate and multiple logistic regression analyses of plasma CatL, CatK, and CatV levels as independent risk factors of human aneurysmal disease.

Dependent variable: AAA	AAA	Mean	SD	OR	Adjusted OR [*]
CatL (ng/mL)	Controls	0.99	0.70	3.04 (2.14; 4.33) <i>P</i> <0.001	2.42 (1.66; 3.52) <i>P</i> <0.001
	AAA	1.38	0.71		
CatK (×100 pmol/L)	Controls	1.16	.60	0.71 (0.52; 0.96) <i>P</i> =0.028	0.78 (0.54; 1.15) <i>P</i> =0.196
	AAA	1.06	.51		
CatV (×100 pg/mL)	Controls	2.76	4.84	0.95 (0.92; 0.99) <i>P</i> =0.017	0.99 (0.94; 1.04) <i>P</i> =0.761
	AAA	1.92	3.5		

* Adjusted for smoking, hypertension, β -blocker use, low-dose aspirin use, statin use, SBP, age, BMI, and lowest ABI.

Table 3

Univariate and partial correlation analyses of the three cathepsins' potential associations with maximal aortic diameter and lowest ABI.

		Maximal aortic diameter		Lowest ABI	
Pearson's correlation analysis		Univariate	Partial *	Univariate	Partial *
CatL	r	0.29	0.212	-0.22	-0.10
	P-value	<0.001	<0.001	<0.001	0.011
CatK	r	-0.05	-0.019	-0.06	-0.01
	P-value	0.248	0.633	0.11	0.983
CatV	r	-0.11	-0.093	-0.01	0.03
	P-value	0.003	0.021	0.986	0.508

* Adjusted for smoking, hypertension, β -blocker use, low-dose aspirin use, statin use, SBP, age, BMI, and lowest ABI.