RESEARCH LETTER

Serum matrix metalloproteinase-8 level in patients with coronary artery abnormal dilatation

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Introduction Coronary artery abnormal dilation (CAAD) is a rare pathology of the coronary arteries with an incidence range of 0.15%–5.3% among patients undergoing coronary angiography.[1] CAAD includes 2 phenotypes: coronary artery aneurysm (CAA) and coronary artery ectasia (CAE). CAA is defined as a focal dilatation of the coronary artery wall with a diameter of 1.5 times larger than the adjacent normal segment. The term CAE describes similar but more diffused lesions.[2]

It is generally considered a benign condition, despite the potential risk of rupture, compression of the surrounding structures, and possible thromboembolic events.[2] The most crucial pathology responsible for the occurrence of CAAD is atherosclerosis, which is widely recognized among patients with cardiovascular diseases.[3] Less common causative factors include Kawasaki disease, diagnostic or interventional coronary angiography, congenital malformation, connective tissue disease, inflammatory and infectious arteritis.[4] CAAD is characterized by vascular remodeling with extensive destruction of the musculoelastic elements, including damage of elastin fibers and reduction in the number of smooth muscle cells.[5] Previous studies have shown that proteolytic enzymes, including metalloproteinases (MMPs), may play a vital role in the pathogenesis of CAE.[6] MMP-8 disturbs the balance between collagen synthesis and its degradation, contributing to vascular remodeling.[7]

Our study aimed to evaluate the plasma levels of MMP-8 in CAAD patients without any angiographically assessed stenosis of coronary arteries in comparison with those with stable angina and obstructive coronary artery disease (CAD) and control subjects with angiographically normal coronary arteries (NCA).

Patients and methods **Study design and patient selection** The presented data result from a sub-analysis of the study previously published by the authors.[8] From the overall group of patients with CAAD, we selected those without any angiographically assessed stenosis (CAAD group, n = 17) and compared them with patients with CAD (CAD group, n = 50) and patients with NCA (control group, n = 50) (Supplementary material, *Figure S1*).

CAAD was defined as a diffuse or focal dilatation of the coronary artery with a diameter 1.5 times larger than that of the adjacent normal segment. The angiographic criteria for CAD included coronary artery stenosis above 90% or intermediate stenosis (50%–90%) with documented ischemia or hemodynamically significant, defined as either fractional flow reserve equal to or lower than 0.80 or an instantaneous wave-free ratio equal to or lower than 0.89. All patients in the control group presented with normal electrocardiography and echocardiography and had no evidence of ischemia during noninvasive stress tests. The exclusion criteria were: (1) acute coronary syndrome; (2) elevated troponin I or creatine kinase levels; (3) history of severe hepatic and renal dysfunction; (4) leukemia, leukopenia, thrombocytopenia, or persistent inflammatory and malignant diseases; (5) systemic diseases of connective tissue; (6) interferon treatment; (7) no informed consent.

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of

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TABLE 1 Baseline characteristics

Baseline data	CAAD group (n = 17)	CAD group (n = 50)	Control group (n = 50)
Male sex	13 (76.5)	40 (80.0)	40 (80.0)
Age, y	64.5 (8.5)	66.4 (7.9)	63.5 (10.1)
BMI, kg/m^2	31.1 (4.3)	28.6 (4.8)	29.1 (6.1)
Previous MI	0[a]	19 (38.0)[a,c]	0[c]
Previous PCI	0[a]	27 (54.0)[a,c]	0[c]
Previous CABG	0[a]	6 (12.0)[a,c]	0[c]
Hypertension	14 (82.3)	46 (92.0)[c]	39 (78.0)[c]
Heart failure	7 (41.2)	19 (38.0)	12 (24.0)
LVEF, %	52.1 (10.7)	52.8 (10.6)	55.6 (8.6)
Diabetes mellitus	6 (35.3)	14 (28.0)	13 (26.0)
Hyperlipidemia	14 (82.3)	40 (80.0)	34 (68.0)
Cigarette smoking	6 (35.3)	18 (36.0)	15 (30.0)
Aortic aneurysm	4 (23.5)	9 (18.0)	8 (16.0)
CKD class ≥2	3 (17.6)	7 (14.0)	4 (8.0)
Drug administration			
Statin	16 (94.1)	49 (98.0)[c]	41 (82.0)[c]
CCB	6 (35.3)	20 (40.0)	20 (40.0)
β-Blocker	16 (94.1)	46 (92.0)[c]	36 (72.0)[c]
Aspirin	15 (88.2)[b]	46 (92.0)[c]	27 (54.0)[b,c]
Clopidogrel	3 (17.6)[a,b]	35 (70.0)[a,c]	0[b,c]
ACEI/ARB	15 (88.2)	45 (90.0)	39 (78.0)
Laboratory tests			
LDL cholesterol, mmol/l	1.5 (0.6)	2.2 (0.8)	2.4 (0.9)
CRP, mg/l	2.5 (2.25–2.35)	2.9 (2.8–3.0)	2.1 (1.8–2.9)
MMP-8, pg/ml	100.6 (22.1–28.1)[a,b]	22.1 (12.4–42.1)[a]	28.7 (11.23–56.8)[b]

Continuous variables are presented as mean (SD) or median (interquartile range), and categorical variables as number (percentage).

a Significant difference (P <0.05) between Group 1 and Group 2

b Significant difference (P <0.05) between Group 1 and Group 3

c Significant difference (P <0.05) between Group 2 and Group 3

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CAAD, coronary artery abnormal dilation; CABG, coronary artery bypass graft; CAD, coronary artery disease; CCB, calcium channel blocker; CKD, chronic kidney disease; CRP, C-reactive protein; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MMP-8, matrix metalloproteinase-8; PCI, percutaneous coronary intervention

Poznan University of Medical Sciences (985/18, approval on Oct 11, 2018).

ELISA analysis EDTA blood samples (10 ml) were collected from all patients on the first day after the cardiac catheterization procedure and were processed within 30 minutes of collection. The samples were centrifuged at 1300 × g for 15 minutes at room temperature. The supernatant was stored at –80 °C.

Measurements were performed in batches. MMP-8 levels were determined using an ELISA sandwich kit (Human MMP-8 ELISA Kit Catalogue Number EHMMP8, Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States) according to the supplier's instructions. Briefly, 100 µl of 10 times diluted plasma were incubated in anti–MMP-8 antibody precoated wells. After washing and aspiration, the samples were incubated with a biotin-conjugated anti–MMP-8 antibody. The streptavidin-HRP solution was added to each well. The quantity of peroxidase bound to each well was determined by adding tetramethylbenzidine. The reaction was stopped, and the resultant color was read at 450 nm in an Epoch Microplate Spectrophotometer (Biotek, Winooski, Vermont, United States). Concentration of the analyzed protein in the sample was determined by interpolation from the standard curve.

Statistical analysis All continuous variables were presented as means (SD) for normal distribution or medians (interquartile range) for non-normal distribution. The normality of the distribution of variables was tested using the Shapiro–Wilk test. Categorical variables were presented as counts and percentages or frequencies. The significance of differences between the mean values of the continuous data was assessed using a one-way analysis of variance (ANOVA) for multiple comparisons. The post hoc Tukey honestly significant difference test was performed to make all pairwise comparisons between groups. The Kruskal–Wallis test with the Dunn Bonferroni–Hochberg post-hoc analysis was used to compare continuous variables with a distribution deviating from the normal. The frequency of occurrence of categorized variables was calculated using the χ^2 test. To indicate differences between 2 groups after the χ^2 test, multiple column comparisons (RxC) with the Benjamini-Hochberg correction were applied. We used PQStat Software (PQStat v.1.8.0.476, Poznań, Poland) for statistical analysis.

Results Baseline characteristics of the CAAD, CAD, and control groups are given in TABLE 1. The 3 groups were similar with regard to age, sex, body mass index, and most other cardiovascular risk factors and comorbidities (P >0.05). In line with the characteristics of the studied groups, CAAD and control groups did not have a history of myocardial infarction and coronary interventions, that is, percutaneous coronary intervention and coronary aortic bypass surgery. A significantly lower prevalence of hypertension was observed in the control vs CAD group (P = 0.049). Similarly, statins and β-blockers were significantly less often used in the control than in the CAD group (P = 0.008 and P = 0.009, respectively). CAD patients were treated with clopidogrel significantly more often than CAAD (P <0.001) and control patients (P <0.001). Aspirin was used significantly less frequently in the control group than in CAD (P <0.001) and CAAD patients (P = 0.008).

ELISA measurements revealed significant differences in the level of MMP-8 between the groups (Supplementary material, *Figure S2*). The plasma level of MMP-8 was significantly

higher in the CAAD group than in the CAD group (P <0.001) and control group (P <0.001)

Discussion Our study focused on evaluating the role of matrix metalloproteinase-8 in the pathogenesis of aneurysmal dilatation of the coronary arteries in patients without significant stenosis. We demonstrated that the plasma levels of MMP-8 were significantly higher in the CAAD group than in the CAD and control groups.

CAAD likely represents an excessive expansive vascular remodeling most often in response to atherosclerotic plaque.[3] By excluding patients with angiographically assessed coronary stenosis from the CAAD group, we selected individuals with a potentially different etiology of vessel remodeling.

It is currently unclear whether MMPs substantially contribute to CAAD formation. However, available data support this statement, reporting a significant correlation between the synthetic phenotype of smooth muscle cells and different MMPs.[9] MMPs are known to play an established role in the pathogenesis, progression, and rupture of an abdominal aortic aneurysm (AAA).[9] Previous studies showed that the formation of AAA leads to a pathophysiological change in extracellular matrix (ECM) composition caused by excessive activity of various proteolytic enzymes, especially MMPs.[10] A similar structure of the aortic and the coronary artery wall suggests that ECM degradation is also one of the pathomechanisms of CAAD formation.

Biomechanical[11] and clinical[12] studies showed that the mechanical strength of a vessel wall depends on the structural collagen network in the media and adventitia. One of the major risk factors for aneurysm growth and rupture is the enhanced turnover of collagen, especially collagen type I and III. Abdul-Hussien et al[13] revealed increased collagen I turnover correlated with MMP-8. Moreover, the authors identified a 3-fold increase in MMP-8 in growing and ruptured AAAs, indicating a significant role of this metalloproteinase in the aortic wall degradation. Wilson et al[14] showed that MMP-8 significantly increased in AAA and correlated positively with intact collagen III and degraded collagen I.[14] MMP-8, also known as collagenase-2, has a solid proteolytic effect on matrix proteins, such as fibrillar collagens, especially collagen type I, laminin, fibronectin, fibromodulin, and many other proteins.[7] It is produced mainly by neutrophils, however, as recently discovered, also by endothelial and smooth muscle cells.[7] Liu et al[15] revealed that the total MMP activity was significantly higher in the CAE group than in the control group. In addition, excessive degradation of elastin fibers and type III collagen and a reduction in total collagen volume were demonstrated.

In summary, the present study showed an association of MMP-8 with CAAD. Further investigation is needed to evaluate the involvement of MMP-8 and other MMPs in CAAD pathogenesis.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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