Original paper

Temporal changes in biomarker levels and their association with the early degeneration stage of transcatheter aortic valves in ^{18}F-fluorodeoxyglucose and ^{18}F-sodium fluoride positron emission tomography studies

Danuta Sorysz[1,2], Artur Dziewierz[1,2], Katarzyna Gawlik[3], Marta Opalińska[4,5], Anna Sowa Staszczak[4,5], Anna Grochowska[6], Krzysztof Piotr Malinowski[7,8], Natalia Maruszak[1], Maciej Bagieński[9], Dariusz Dudek[8]

[1]2nd Department of Cardiology, Institute of Cardiology, Jagiellonian University Medical College, Krakow, Poland
[2]Clinical Department of Cardiology and Cardiovascular Interventions, University Hospital, Krakow, Poland
[3]Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland
[4]Clinical Department of Endocrinology, Oncological Endocrinology and Nuclear Medicine, University Hospital, Krakow, Poland
[5]Department of Endocrinology, Jagiellonian University Medical College, Krakow, Poland
[6]Department of Radiology, University Hospital, Krakow, Poland
[7]Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College, Krakow, Poland
[8]Center for Digital Medicine and Robotics, Jagiellonian University Medical College, Krakow, Poland
[9]Intensive Cardiac Care Unit, University Hospital, Krakow, Poland

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Abstract

Introduction: As transcatheter aortic valve implantation (TAVI) indications expand, understanding the valve degeneration process and potential influencing biomarkers becomes increasingly important.

Aim: To investigate temporal changes in biomarker levels and their potential association with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) and ^{18}F-sodium fluoride (^{18}F-NaF) uptake, assessed using positron emission tomography/computed tomography (PET/CT) studies as markers for native aortic annulus calcifications and early-stage TAVI valve degeneration.

Material and methods: A total of 71 TAVI patients underwent blood sampling and transthoracic echocardiography at baseline (pre-TAVI) and 6, 12, 18, and 24 months after the procedure. PET/CT using ^{18}F-NaF and ^{18}F-FDG was performed at 6 and 24 months. Serum levels of matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-9 (MMP-9), and osteopontin (OPN) were measured. In addition, plasma levels of osteoprotegerin (OPG), lipoprotein a (Lp(a)), and oxidized LDL (ox-LDL) were assessed.

Results: Finally, 31 patients (median age: 84.0 years) completed the study. Valve function improved after TAVI and remained stable during follow-up. Over 24 months, OPN levels decreased (p = 0.010), while MMP-3 and MMP-9 levels increased (p = 0.046 and p = 0.041). MMP-3 and MMP-9 showed multiple positive correlations across time points. OPN, ox-LDL, and OPG demonstrated significant negative correlations with follow-up effective orifice area index and effective orifice area (EOA). No significant correlations were found between biomarkers and PET/CT uptake.

Conclusions: Significant biomarker changes over 24 months and negative correlations with EOA suggest potential roles in aortic valve function. However, no correlations between biomarkers and PET/CT results were observed.

Key words: biomarkers, lipoprotein a, metalloproteinases, osteopontin, osteoprotegerin, positron emission tomography, transcatheter aortic valve implantation, valve degeneration, valve durability.

Corresponding author:
Danuta Sorysz MD, PhD, 2nd Department of Cardiology, Institute of Cardiology, Jagiellonian University Medical College, Krakow, Poland, phone: +48 12 400 2250, e-mail: danuta.sorysz@uj.edu.pl
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Summary

This study investigated temporal changes in biomarker levels and their potential association with positron emission tomography/computed tomography (PET/CT) uptake markers in transcatheter aortic valve implantation (TAVI) patients over 24 months. Significant changes were observed in several biomarkers, with osteopontin levels decreasing and matrix metalloproteinase-3 and matrix metalloproteinase-9 levels increasing over time, while negative correlations were found between some biomarkers and measures of valve function. However, no significant correlations were identified between the measured biomarkers and PET/CT uptake results, suggesting a complex relationship between biochemical markers and imaging indicators of valve degeneration in TAVI patients.

Introduction

Transcatheter aortic valve implantation (TAVI) has become an established minimally invasive treatment for severe aortic stenosis, increasingly used in lower-risk and younger patients [1–4]. As TAVI use expands, there is a pressing need to understand the factors influencing bioprosthesis degeneration. While the pathophysiology of this process remains poorly understood, it likely mirrors aortic stenosis development, beginning with localized valve inflammation leading to leaflet calcification [5].

Recent research focused on the potential role of inflammation and calcification biomarkers in bioprosthesis degeneration [5–7]. Identifying blood biomarkers that can predict patients at risk of, or experiencing faster progression of, bioprosthesis degeneration could have significant clinical value [7]. Matrix metalloproteinase-3 (MMP-3) is a key inflammatory factor secreted by cardiac fibroblasts and macrophages. It activates other metalloproteinases, such as matrix metalloproteinase-9 (MMP-9), and pro-inflammatory mediators [8, 9]. Pathological effects of MMP-3 include endothelial injury and inflammatory cell accumulation in the cardiovascular system [8, 9]. MMP-9 secreted by cardiomyocytes, endothelial cells, neutrophils, and macrophages serves as a marker of inflammation and cardiac remodeling. It promotes cardiac fibroblast proliferation and migration through various mechanisms, including the release of two biologically active peptides from osteopontin (OPN) cleavage [10, 11]. Serum MMP-9 levels reflect arterial inflammation and have been associated with coronary artery disease, arterial hypertension, cardiovascular events, and mortality [10]. Oxidized low-density lipoprotein (ox-LDL) refers to heterogeneous oxidative modifications of LDL lipid components and apolipoprotein B (apoB), the primary protein in LDL particles [12–14]. Ox-LDL increases the expression of the matrix-degrading enzymes MMP-1 and MMP-3 and enhances MMP-9 production through macrophage stimulation [13]. Elevated ox-LDL levels can trigger arterial inflammation, potentially leading to atherosclerosis or heart disease. Furthermore, plaque instability in atherosclerotic lesions is also associated with high ox-LDL levels [14].

Lipoprotein (a) (Lp(a)) is a prevalent genetic risk factor for cardiovascular disease and calcific aortic valve disease [15, 16]. Its significance is reflected in the position paper of the European Society of Atherosclerosis [16]. Like LDL, Lp(a) contains apoB, but circulates in smaller quantities. Lp(a) can induce arterial wall inflammation, and high levels accelerate coronary artery calcification. Similarly, elevated Lp(a) promotes aortic valve stenosis by triggering inflammatory and calcifying gene expression in valvular interstitial cells [15]. Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor family, is considered an anti-calcifying protein [17]. It regulates osteoblast and osteoclast activity, and directly affects endothelial and vascular smooth muscle cells [18]. Studies have shown elevated serum OPG levels in aortic stenosis patients, correlating with increased mortality [19]. Osteopontin, encoded by the OPN gene, is a multifunctional protein contributing to the inflammatory microenvironment characteristic of atherosclerosis. Several studies have demonstrated that OPN levels increase with atherosclerosis progression and calcification, indicating a pro-calcific role [20, 21]. Elevated OPN levels have also been associated with cardiac remodeling progression, making it a valuable biomarker for cardiovascular disease and events [22].

Previous studies have validated the use of PET-CT in imaging aortic stenosis degeneration, utilizing both FDG and NaF tracers to assess calcification. Notably, Dweck *et al.* demonstrated the potential of NaF-labelled PET-CT in evaluating the degeneration of aortic bioprostheses, including both surgical and TAVI valves [23]. Given the established utility of NaF- and FDG-labelled PET-CT in assessing aortic degeneration and calcification, our study aimed to investigate the relationship between biomarker levels and the activity of these isotopes at the site of implanted TAVI valves 6 and 24 months after implantation. By examining these relationships over time, we hope to gain insights into the progression of TAVI valve degeneration and identify potential predictive markers for long-term valve durability.

Material and methods

Between July 2017 and January 2020, 71 consecutive patients with symptomatic severe aortic stenosis, eligible for TAVI, were prospectively enrolled in a single-center observational study [24]. The research protocol included a baseline visit (2 days before TAVI) and follow-up clinical visits at 1, 6, 12, and 24 months post-procedurally.

Each visit involved blood sample collection and transthoracic echocardiography (TTE). PET-CT and transesophageal echocardiography (TEE) were scheduled for the 6-month and 24-month follow-up visits. However, due to the SARS-CoV-2 pandemic, some 24-month PET-CT/TEE examinations and clinical follow-ups with TEE were postponed or cancelled for this high-risk population. Additionally, 8 patients developed subclinical valve thrombosis during follow-up, necessitating postponement of their PET-CT scans until resolution. The final PET-CT results were compared with initial data and corelated with biomarker levels.

Biochemistry

Fasting serum and plasma (EDTA) samples were collected before TAVI and at 6, 12, and 24 months after the procedure. Samples were centrifuged at 2000 *g* for 10 min, aliquoted, and stored at –80°C until analysis. Serum MMP-3, MMP-9, and OPN were measured. In addition, plasma OPG, Lp (a), and ox-LDL were assessed. Commercial ELISA kits for MMP-3 (R&D Systems, DMP300), MMP-9 (R&D Systems, DMP900), OPN (R&D Systems, DOST00), OPG (BioVendor RD194003200), Lp(a) (Mercodia 10-1106-01) and ox-LDL (Mercodia 10-1143-01) were used according to the manufacturers' instructions. All measurements were performed at the Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland, using a BioTek ELX808 Absorbance Microplate Reader.

Echocardiography

All patients underwent TTE at baseline and during follow-up, including two-dimensional, three-dimensional, and Doppler imaging. Image acquisition settings were optimized for maximum quality. For enhanced visualization of TAVI valve leaflets, two-dimensional and three-dimensional TEE were performed at 6 months and the final follow-up. All examinations were conducted using Vivid E9 and Vivid E95 systems (GE Healthcare, Waukesha, WI, USA). Post-processing evaluation employed a dedicated workstation (EchoPAC, GE Healthcare). Linear measurements were utilized virtual calipers. TTE examinations and measurements adhered to recommendations for assessing aortic stenosis and TAVI valves.

Positron emission tomography/computed tomography (PET/CT)

Electrocardiography (ECG)-gated PET/CT scans of the aortic valve were performed using ^{18}F-fluorodeoxyglucose (FDG) and ^{18}F-sodium fluoride (NaF) on a GE DISCOVERY 690 VCT scanner (GE Healthcare, Milwaukee, WI, USA). Radiopharmaceuticals were injected intravenously at 4 MBq per kg body mass. For ^{18}F-FDG, patients rested for 60 min after the injection. The protocol included an attenuation correction CT scan (non-enhanced, ECG-gated, low-dose 120 kV with modulated rays, 80–220 mA), followed by PET imaging covering one bed position (15 cm) centered over the valve in 3D mode for 16 min. PET data reconstruction used GE's Vue Point FX algorithm (matrix size 128 × 128; for static images: 24 subsets, 2 iterations with Cardiac 3D filter, 12 mm cut-off; for gated images: 24 subsets, 5 iterations with Cardiac 3D filter, 4.3 mm cut-off) producing 8-slice CT-gated PET images. Corrections were applied for attenuation, dead time, scatter, and random coincidences. PET image short-axis thickness was 3.3 mm. CardIQ Fusion PET software was used to analyze the fused PET/CT images. The detailed method for evaluating PET parameters of TAVI valves has been described previously [24]. In brief, measurements were taken during diastole for more reliable results. Three-dimensional multiplanar reconstruction visualized the valve in short-axis coaxial view. Regions of interest (ROIs) were drawn inside and outside the stent at closed leaflets level and two adjacent cross sections. The "inner area" was defined as the area inside the valve (including the leaflets but excluding the stent and aortic annulus). The "outer area" included valve leaflets, aortic annulus, and native leaflets. Three independent nuclear medicine specialists took measurements, which were then averaged. Mean and maximum standardized uptake values (SUV) were calculated for three cross-sections of inner and outer regions, then averaged for analysis. To reduce the variability of results, SUVs were normalized by calculating tumor-to-background ratio (TBR) using SUV mean of mediastinal blood pool (MBPS).

Statistical analysis

Categorical variables are presented as numbers and percentages, while continuous variables are expressed as either mean ± SD or median (interquartile range (IQR)), as appropriate. The Shapiro-Wilk test was used to assess normality, and the Levene test was applied to evaluate the equality of variances. For normally distributed variables, differences between baseline and follow-up parameters were compared using mixed effect models. The Pearson χ^2 test or Fisher exact test was used to compare categorical variables. Pearson correlation coefficient values were computed to evaluate the association between laboratory, echocardiographic, and PET-CT parameters. Two-sided p-values < 0.05 were considered significant. All analyses were performed using R 4.2.3 software (R Foundation for Statistical Computing, Vienna, Austria), with the packages lme4, version 1.1–30, and emmeans, version 1.8.2, as well as IBM SPSS Statistics version 29.0.0 (IBM Corp, Armonk, NY, USA).

Results

A total of 71 TAVI patients were included in the study, with a median age of 84.0 years (IQR: 80.0–87.0) and 59.1% being female [24]. The cohort exhibited typical

risk factors for this population, including chronic kidney disease (35.5%), arterial hypertension (87.3%), diabetes mellitus (39.4%), and previous myocardial infarction (35.2%). Five types of aortic valves were used, with the Medtronic Evolut R being the most common (71%), followed by the Boston Scientific Accurate Neo (16.1%). Other valves used were the Edwards Lifesciences SAPIEN 3, Medtronic Evolut PRO, and St. Jude Medical Portico. The mortality rates were 9.6% at 1 month, 13.7% at 6 months, and 45% at 68 months.

The mean left ventricular ejection fraction (LVEF) was 55.6 ±3.0%, with an aortic valve area (AVA) of 0.6 ±0.1 cm², peak gradient (PG) of 85.3 ±3.1 mm Hg, and mean pressure gradient (MPG) of 51.1 ±2.0 mm Hg. TAVI resulted in a significant increase in AVA to 1.6 ±0.1 cm² after 1 month ($p < 0.001$), and a decrease in PG to 17.0 ±3.3 mm Hg and MPG to 9.4 ±2.1 mm Hg ($p < 0.001$ for both). No significant changes in LVEF were observed. These improvements in AVA, PG, and MPG were maintained over a 24-month follow-up period.

Out of the 71 patients, baseline and final follow-up ^{18}F-NaF and ^{18}F-FDG PET/CT scans were performed on 31 patients. The median (IQR) time interval from the baseline PET scan, conducted 6 months after TAVI, to the

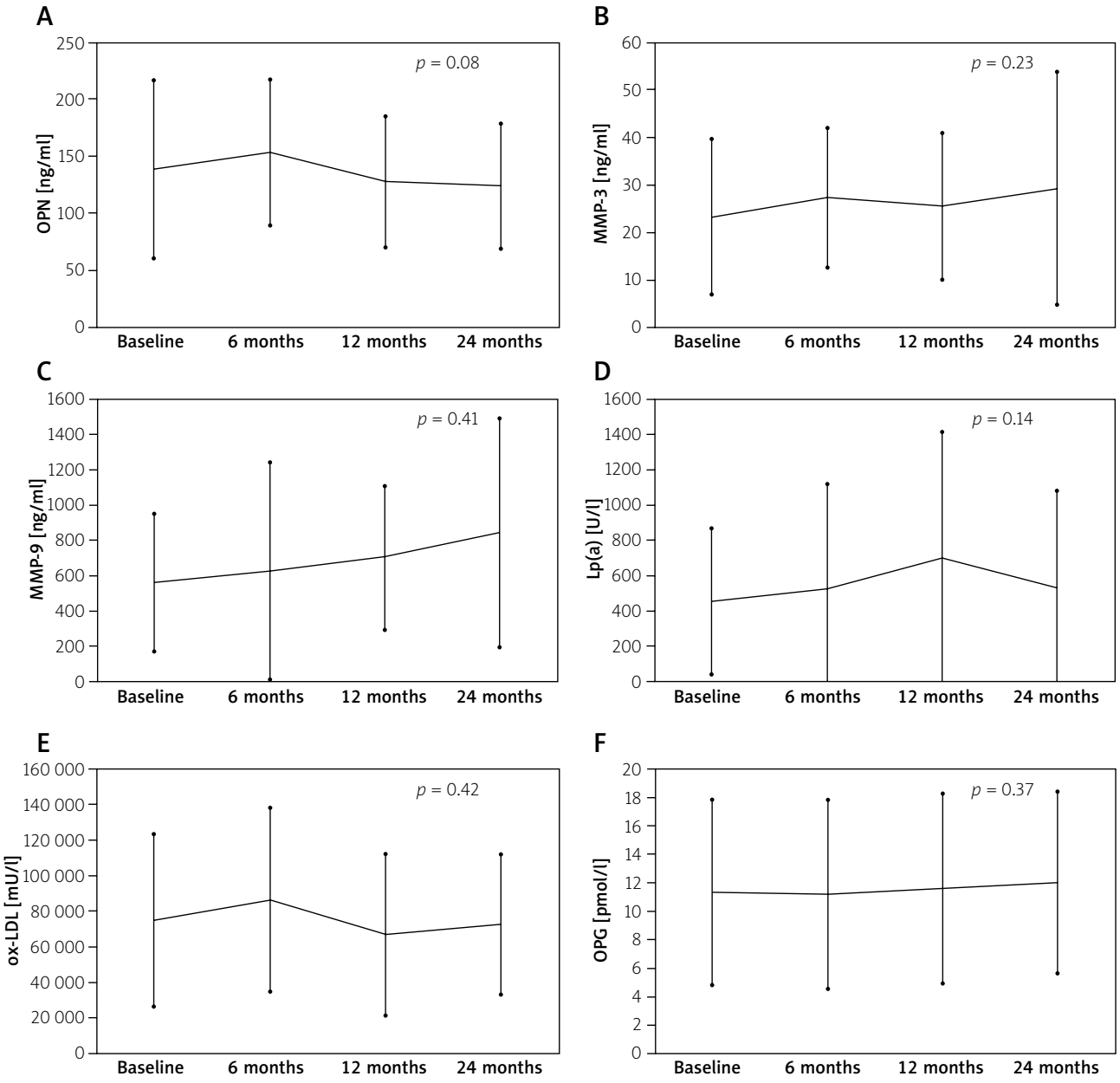

Figure 1. Changes in levels of osteopontin (OPN, **A**), matrix metalloproteinase-3 (MMP-3, **B**), matrix metalloproteinase-9 (MMP-9, **C**), lipoprotein(a) (Lp(a), **D**), oxidized low-density lipoprotein (ox-LDL, **E**), and osteoprotegerin (OPG, **F**) during follow-up

follow-up PET/CT scan was 18.1 (15.5–21.8) months for ^{18}F-NaF and 18.5 (15–26.7) months for ^{18}F-FDG. As noted previously, the analysis of ^{18}F-FDG and ^{18}F-NaF PET/CT scans revealed varying patterns of uptake and inflammation in valve segments over time. For ^{18}F-FDG, microinflammation showed similar or increasing values at follow-up. The inner areas of the bottom and middle valve segments showed comparable microinflammation, but there was an increase in SUVmean in the outer area of the middle segments. Notably, significant differences in corrected SUVmax ^{18}F-FDG (TBR-RA) were observed between baseline and follow-up. In contrast, ^{18}F-NaF PET/CT showed no differences in absolute SUVmax or SUVmean between inner and outer areas, regardless of the mapping level, and both absolute and corrected values showed no significant differences between baseline and follow-up.

Significant changes in several biomarkers over a 24-month period were noted. OPN levels decreased significantly from baseline (139.1 ±78.1) to 24 months (124.2 ±54.7, p = 0.010), with a trend suggesting a possible linear effect of time (p = 0.08) – Figure 1 A. MMP-3 levels increased significantly from baseline (23.4 ±16.3) to 24 months (29.4 ±24.5, p = 0.046), although multivariate and univariate tests indicated no significant overall effect of time – Figure 1 B. MMP-9 levels showed a significant linear effect of time (p = 0.041) and increased from baseline (559.1 ±391.6) to 24 months (843.2 ±650.5, p = 0.016) – Figure 1 C. In contrast, no significant changes were observed in Lp(a) levels (baseline: 453.4 ±415.2, 24 months: 530.1 ±553.6, p = 0.24), ox-LDL levels (baseline: 75136.6 ±48586.9, 24 months: 72728.4 ±39424.3, p = 0.55), or OPG levels (baseline: 11.4 ±6.5, 24 months:

Table I. Correlation coefficients for association between laboratory results and follow-up echocardiographic parameters

Parameter	Echo parameters				
	LVEF	EOA	EOAi	PG	MPG
OPN:					
Baseline	0.265	–0.591**	–0.908*	0.414	0.427
6 months	0.167	–0.079	0.371	0.300	0.300
12 months	–0.024	–0.274	–0.968**	0.333	0.386
24 months	–0.158	0.420	0.366	–0.194	–0.131
Lp(a):					
Baseline	0.326	–0.078	–0.295	0.220	0.183
6 months	0.094	–0.006	–0.269	–0.043	–0.060
12 months	0.166	0.181	0.031	0.182	0.144
24 months	0.266	–0.235	–0.909*	0.379	0.404
ox-LDL:					
Baseline	0.520*	–0.189	0.024	0.129	0.121
6 months	0.470*	–0.508*	–0.629	0.152	0.170
12 months	0.439	–0.249	–0.136	0.165	0.175
24 months	–0.065	–0.108	0.135	–0.076	–0.099
MMP-3:					
Baseline	0.007	0.018	0.057	0.028	0.069
6 months	0.131	0.287	0.055	–0.119	–0.086
12 months	0.280	0.063	0.291	0.131	0.181
24 months	0.277	0.204	0.426	0.304	0.327
MMP-9:					
Baseline	0.253	0.046	–0.197	0.322	0.340
6 months	0.359	0.228	–0.122	0.173	0.189
12 months	0.364	–0.111	0.116	0.299	0.275
24 months	0.454	–0.043	–0.263	0.291	0.306
OPG:					
Baseline	–0.147	–0.277	–0.311	0.162	0.165
6 months	–0.016	–0.539*	–0.487	0.439	0.478*
12 months	–0.168	–0.515*	–0.466	0.400	0.425
24 months	0.004	–0.414	–0.506	0.179	0.185

*EOA – effective orifice area, EOAi – effective orifice area index, LVEF – left ventricular ejection fraction, Lp(a) – lipoprotein(a), MMP-3 – matrix metalloproteinase-3, MMP-9 – matrix metalloproteinase-9, MPG – mean pressure aortic gradient, OPG – osteoprotegerin, OPN – osteopontin, ox-LDL – oxidized low-density lipoprotein, PG – peak aortic gradient. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).*

12.1 ±6.4, $p = 0.43$) over the study period – Figures 1 D–F. Baseline ox-LDL levels showed a strong positive correlation with MMP-9 levels at both 12 months ($r = 0.518$, $p < 0.001$) and 24 months ($r = 0.337$, $p = 0.042$), suggesting a potential relationship between ox-LDL and MMP-9 activity. Additionally, MMP-3 and MMP-9 levels demonstrated multiple positive correlations across various time points, with the strongest correlation observed at 24 months ($r = 0.455$, $p = 0.005$), indicating a potential interaction or common regulatory mechanism.

As shown in Table I, baseline and 12-month OPN demonstrated significant negative correlations with follow-up effective orifice area index (EOAi). Ox-LDL obtained at baseline and 6 months showed significant positive correlations with follow-up LVEF, but negatively correlated with EOA. Six- and 12-month OPG exhibited significant negative correlations with EOA, and a positive correlation with MPG, suggesting its potential role in aortic valve function over time. Conversely, Lp(a), MMP-3, and MMP-9 showed weak or non-significant correlations with follow-up echocardiographic parameters.

No strong correlations between the assessed biomarkers and ^{18}F-FDG or ^{18}F-NaF uptake in PET/CT scans at 24 months, using either absolute (Tables II and III) or standardized (data not shown) SUV values, were confirmed. Additionally, no significant associations were found between changes (absolute and relative) in biomarker values during follow-up and PET/CT scan uptake results (data not shown).

Discussion

The mechanisms of TAVI valve degeneration remain incompletely understood. Therefore, this study aimed to evaluate the potential association between biomarkers

Table II. Correlation coefficients for association between laboratory results and follow-up ^{18}F-sodium fluoride positron emission tomography/computed tomography (^{18}F-NaF PET/CT) scans

Parameter	SUV max (overall)	SUV mean (overall)	SUV max (inner)	SUV mean (inner)	SUV max (outer)	SUV mean (outer)
OPN:						
Baseline	–0.019	0.067	0.077	0.155	–0.075	–0.010
6 months	0.028	–0.133	0.063	–0.113	0.005	–0.135
12 months	0.223	0.081	0.261	0.200	0.193	0.018
24 months	–0.202	–0.349	–0.143	–0.205	–0.219	–0.409*
Lp(a):						
Baseline	0.100	0.096	0.029	–0.061	0.133	0.210
6 months	0.198	0.199	0.164	0.065	0.202	0.287
12 months	0.212	0.170	0.188	0.021	0.212	0.299
24 months	0.049	0.061	0.022	–0.019	0.064	0.131
ox-LDL:						
Baseline	–0.164	–0.128	–0.196	–0.232	–0.134	–0.032
6 months	–0.164	–0.209	–0.088	–0.168	–0.192	–0.197
12 months	–0.105	–0.145	–0.123	–0.132	–0.082	–0.111
24 months	–0.086	–0.154	–0.055	–0.101	–0.098	–0.179
MMP-3:						
Baseline	–0.222	–0.185	–0.266	–0.170	–0.181	–0.191
6 months	–0.257	–0.282	–0.274	–0.239	–0.226	–0.271
12 months	–0.180	–0.108	–0.228	–0.090	–0.135	–0.098
24 months	0.014	0.014	–0.082	–0.033	0.073	0.065
MMP-9:						
Baseline	–0.062	0.183	–0.012	0.186	–0.087	0.166
6 months	0.125	0.209	0.150	0.216	0.108	0.220
12 months	–0.057	–0.218	–0.120	–0.242	–0.011	–0.135
24 months	–0.114	–0.065	–0.155	–0.121	–0.078	0.009
OPG:						
Baseline	–0.317	–0.333	–0.256	–0.279	–0.333	–0.358*
6 months	–0.107	–0.080	–0.031	–0.003	–0.143	–0.124
12 months	–0.338	–0.127	–0.222	–0.023	–0.385	–0.239
24 months	–0.157	–0.135	–0.077	–0.132	–0.196	–0.134

*SUV – standardized uptake value; others see Table I. *Correlation is significant at the 0.05 level (2-tailed).*

Table III. Correlation coefficients for association between laboratory results and follow-up ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) scans

Parameter	SUV max (overall)	SUV mean (overall)	SUV max (inner)	SUV mean (inner)	SUV max (outer)	SUV mean (outer)
OPN:						
Baseline	–0.138	–0.053	–0.112	–0.084	–0.148	–0.012
6 months	–0.134	–0.232	–0.156	–0.223	–0.102	–0.229
12 months	–0.005	–0.105	–0.078	–0.141	0.056	–0.051
24 months	0.012	–0.079	0.066	–0.074	–0.034	–0.078
Lp(a):						
Baseline	0.155	0.239	0.127	0.261	0.165	0.211
6 months	–0.027	0.150	0.048	0.177	–0.088	0.116
12 months	0.179	0.287	0.067	0.276	0.256	0.283
24 months	0.023	0.078	–0.020	0.069	0.057	0.091
ox-LDL:						
Baseline	0.101	0.150	–0.102	0.092	0.265	0.208
6 months	–0.043	–0.007	–0.025	–0.058	–0.055	0.057
12 months	0.133	0.189	–0.129	0.120	0.336	0.243
24 months	0.140	0.023	0.057	–0.001	0.197	0.048
MMP-3:						
Baseline	–0.112	–0.277	–0.062	–0.270	–0.145	–0.275
6 months	–0.032	–0.083	–0.063	–0.106	–0.002	–0.056
12 months	0.077	0.082	0.072	0.034	0.075	0.127
24 months	0.058	0.040	0.078	0.062	0.039	0.006
MMP-9:						
Baseline	–0.141	0.045	–0.019	0.107	–0.233	–0.032
6 months	0.078	0.298	0.103	0.332	0.053	0.246
12 months	–0.074	–0.072	–0.266	–0.127	0.088	–0.012
24 months	–0.037	0.038	–0.028	0.059	–0.045	0.012
OPG:						
Baseline	–0.250	–0.456*	–0.169	–0.449*	–0.298	–0.443*
6 months	–0.116	–0.241	–0.102	–0.241	–0.127	–0.228
12 months	–0.098	–0.365	–0.006	–0.402	–0.165	–0.304
24 months	0.002	–0.266	0.015	–0.265	–0.011	–0.261

*SUV – standardized uptake value; others see Table I. *Correlation is significant at the 0.05 level (2-tailed).*

and the presence of inflammatory features and calcification of the native aortic annulus and TAVI valve leaflets, as assessed by PET-CT using FDG and NaF tracers. Our main finding was a lack of correlation between the studied biomarkers and FDG and NaF activity on PET-CT. However, we observed higher levels of MMP-9 and MMP-3 in patients 24 months after TAVI compared to baseline, although the change in MMP-3 concentration did not reach statistical significance.

Current hypotheses suggest that TAVI valve degeneration may resemble the changes that occur in native valve leaflets, involving initial local inflammation followed by valve calcification in more advanced stages [5]. Dweck *et al*. documented the feasibility of FDG-PET/CT for diagnosing early-stage degeneration and NaF-PET/CT for evaluating aortic valve calcification [23]. Other studies have shown increased FDG uptake in the first month after implantation [25]. Interestingly, at certain stages of progression, calcifications may no longer be metabolically active, resulting in no increased NaF uptake on PET-CT. Our previous study revealed an increase in FDG-PET activity but no change in NaF activity during 24-month follow-up of post-TAVI patients [24]. This finding is notable given that NaF-PET can detect microcalcifications as small as 50 µm in diameter, which is beyond the sensitivity of CT calcium scoring [26]. In the study, we analyzed biomarkers associated with inflammation – Lp(a), MMP-3, MMP-4, OPG – and calcification (OPN and OPG) to further elucidate their role in TAVI valve degeneration.

The literature largely supports the association between elevated Lp(a) levels and cardiovascular risk, including aortic valve and aortic microcalcifications. However, this relationship has been primarily observed in younger populations (aged 40–45) [15]. Our study, which

included older patients, did not find a correlation between Lp(a) levels and NaF uptake on PET-CT. This discrepancy may be due to our older cohort or because NaF-PET/CT only images metabolically active calcifications, while our study group likely included calcifications no longer showing activity. This insight could be crucial for designing studies on new molecules aimed at inhibiting progressive aortic valve degeneration using PET-CT. MMP-9 serum concentration increases may indicate a higher risk of bioprosthetic valve degeneration, as it is a marker of inflammation, proliferation, and tissue/cardiac remodeling [27]. Other biomarkers showed non-significant changes: OPG levels increased while OPN decreased, and Lp(a) and ox-LDL concentrations fluctuated non-linearly. Elevated OPG is consistent with a study conducted by Lis *et al.* [28]. Elevated serum OPG was associated with decreased osteoclastic differentiation in stenotic aortic valves [29]. OPG is a protective factor for the vascular system [30]. Increased OPG production may indicate endothelial damage, smooth muscle cell hypertrophy, or advanced plaque calcification and may represent a compensatory mechanism to prevent further vascular damage. Some studies have indicated that OPG has a strong correlation with cardiovascular diseases [30, 31]. We also observed that MMP-9 concentration changes in 6, 12 and 24 months correlated with vascular inflammation measured by FDG PET/CT. According to the clinical literature, it could reflect both cardiovascular inflammation and other pathophysiological processes [32]. In patients with coronary artery disease, MMP-9 has been correlated with other inflammation biomarkers [33]. A positive relationship between MMP-9 and ox-LDL at 12 months after TAVI was observed, in line with previous studies [34]. This correlation may be due to ox-LDL's proinflammatory properties and its ability to increase MMP-9 secretion by macrophages [13]. Ox-LDL formation is associated with oxidative stress, which also triggers increased MMP-9 levels [34, 35].

Our study has several important limitations to consider. The most significant limitations are the small number of participants and the risk of selection bias. The COVID-19 pandemic unfortunately prevented us from reaching our initial target of 80 patients, further reducing our sample size. Another key limitation is the relatively short follow-up period, which may not have been sufficient to fully capture the progression of TAVI valve degeneration. Furthermore, while innovative, the PET imaging technique we employed has inherent limitations in terms of temporal and spatial resolution. Finally, biomarker analysis was limited to the examination of only a few markers. Additionally, these were not specific to aortic valve degeneration, and their levels may be affected by several factors and comorbidities.

Conclusions

Significant biomarker changes over 24 months and negative correlations with EOA suggest potential roles in aortic valve function. However, no correlations between biomarkers and PET/CT results were observed.

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Ethical approval

The Institutional Review Board of Jagiellonian University Medical College approved this study (approval number 1072.6120.90.2017). All participants provided written informed consent. The study adhered to the ethical principles outlined in the 1975 Declaration of Helsinki.

Conflict of interest

The authors declare no conflict of interest.

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