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Article

Platelet-Related Biomarkers and Efficacy of Antiplatelet Therapy in Patients with Aortic Stenosis and Coronary Artery Disease

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Abstract

The objective of the study was to evaluate the serum biomarkers implicated in the interaction of platelets and endothelium, as well as the efficacy of antiplatelet therapy in patients with aortic stenosis (AS) and coronary artery disease (CAD). A total of 78 adult patients with CAD on aspirin therapy participated in the study, including 49 consecutive patients with AS and 29 control subjects. The analysis included the following serum biomarkers: thrombomodulin (TM), platelet factor 4 (PF4), P-selectin, and CD40L. The efficacy of antiplatelet treatment was evaluated using Verify Now Aspirin (ASPI test) and P2Y12 assay (ADP test). AS patients exhibited increased serum levels of TM (7.64 ± 3.5 ng/mL vs. 6.28 ± 2.1 ng/mL, p = 0.011) and PF4 (25.16 ; Q1:8.3; Q3:29.6 μg/mL vs. 12.85 ; Q1:5.7; Q3:14.5 μg/mL, p = 0.021) compared to the control group. P-selectin and CD40L levels did not differ between groups. There were no differences in platelet aggregation in the ASPI (474.04 ± 66.7 ARU vs. 471.31 ± 56.2 ARU; p = 0.822) or ADP (224.88 ± 46.4 PRU vs. 216.62 ± 29.6 PRU; p = 0.394) tests. Bleeding incidence did not differ significantly between groups. The coexistence of AS in patients with CAD is associated with elevated levels of biomarkers indicative of endothelial damage and platelet activation. However, the efficacy of antiplatelet treatment was independent of the presence of AS.

Keywords: aortic stenosis; platelets; biomarkers; thrombomodulin; platelet factor 4; P-selectin; CD40L; antiplatelet therapy; aspirin.

1. Introduction

Aortic stenosis (AS) is a progressive valvular heart disease that begins with minor fibrocalcific changes in the leaflets and progresses to more pronounced calcification [1]. Aortic valve sclerosis develops gradually, involving endothelial damage, lipid accumulation, and inflammation [2–7]. As the disease progresses, the valve opening becomes more constricted, resulting in turbulent blood flow that adversely impacts hemodynamics. Turbulent blood flow and shear stress have been demonstrated to contribute to endothelial dysfunction and platelet activation [8–12]. Activation of platelets leads to the release of proinflammatory cytokines and chemokines [1,7]. Antiplatelet therapy, a prevalent treatment modality in the management of cardiovascular disease, aims to prevent platelet activation and aggregation, thereby reducing the risk of thrombosis. Current and emerging therapeutic interventions target various platelet receptors, including COX-1, P2Y12, and integrin receptors. Aspirin, a COX-1 inhibitor, has been demonstrated to reduce platelet

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prothrombotic activity, while P2Y12 inhibitors have been shown to block platelet activation via the PI3K/AKT pathway [13,14].

The presence of coronary artery disease (CAD) frequently coincides with AS and in this group of patients antiplatelet therapy is used in the prevention of cardiovascular incidents. However, AS, characterized by excessive platelet activation, may affect the effectiveness of antiplatelet therapy [15]. Conversely, excessive shear stress exerted on the aortic wall can result in significant degradation of high-molecular weight multimers of von Willebrand factor, which has been associated with an elevated risk of bleeding, including gastrointestinal bleeding, as observed in Heyde's syndrome [16,17]. An increasing body of research is evaluating the efficacy and safety of aspirin and P2Y12 receptor inhibitors in the prevention of cardiovascular events among patients with CAD. Several studies have reported a lower risk of bleeding in patients treated with clopidogrel and currently in different clinical settings clopidogrel is used instead of aspirin [18–20]. In contrast, data on the effects of AS on platelet function and the safety of antiplatelet therapy in patients with both CAD and AS are limited and remain an interesting field of research.

The study evaluates the serum biomarkers implicated in the interaction of platelets and endothelium, such as thrombomodulin (TM), platelet factor 4 (PF4), and P-selectin, as well as inflammation-related biomarkers, including CD40L.

TM, an integral membrane protein, is predominantly expressed on the surface of endothelial cells that line blood vessels. It plays a crucial role in the regulation of hemostasis, particularly in the anticoagulant pathway [21]. TM functions as a cofactor for thrombin, a pivotal enzyme in the blood clotting cascade. Upon binding to TM, thrombin's pro-coagulant activity is neutralized. Impaired TM expression has been observed in damaged or dysfunctional endothelium, contributing to vascular thrombosis [22,23].

PF4 is a small cytokine that is released from the alpha granules of activated platelets during platelet aggregation. It plays a multifaceted role in the processes of hemostasis, immunity, and inflammation [24]. Complexes of PF4 have been associated with increased platelet activation, inflammation, and an elevated risk of thrombosis [25].

P-selectin, a cell adhesion molecule, is stored in the Weibel–Palade bodies of endothelial cells and the alpha granules of platelets. P-selectin plays a pivotal role in the initial rolling of leukocytes, particularly neutrophils and monocytes, along the activated endothelium [26]. P-selectin has been shown to promote thrombus formation by facilitating interactions between platelets, leukocytes, and endothelial cells [27].

CD40L is a transmembrane protein that is predominantly expressed on activated T cells, but is also found on platelets, B cells, monocytes, and endothelial cells. The activation of CD40 by CD40L has been demonstrated to stimulate the production of pro-inflammatory cytokines, such as IL-6 and TNF-α [28,29]. This process has also been observed to induce the expression of adhesion molecules and the release of matrix metalloproteinases. When expressed on activated platelets, CD40L has been shown to promote platelet-leukocyte aggregates, endothelial activation, and enhanced thrombus formation [30].

The objective of the present study was to evaluate the serum biomarkers implicated in the interaction of platelets and endothelium, such as TM, PF4, P-selectin, and CD40L. The study also sought to determine the efficacy of antiplatelet therapy in patients with AS and CAD by measuring platelet aggregation.

2. Results

2.1. Clinical and Laboratory Characteristics

A comparative evaluation of clinical and biochemical characteristics demonstrated statistically significant differences between patients with AS and the control group. Patients with AS exhibited a lower prevalence of tobacco use (14.9% vs. 41.4%, $p = 0.01$). Moreover, these patients were characterized by a higher NYHA class (2.51 ± 0.8 vs. 1.59 ± 1.1, $p < 0.001$). Additionally, patients with

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AS demonstrated elevated serum creatinine levels (1.13 ± 0.6 mg/dL vs. 0.9 ± 0.1 mg/dL; p = 0.012) and reduced estimated glomerular filtration rate (64.91 ± 22.2 mL/min vs. 75.21 ± 15.3 mL/min) compared to controls. The clinical and the laboratory characteristics are shown in Table 1 and Table 2.

Table 1. Baseline clinical characteristics. AS- aortic stenosis; CAD-coronary artery disease; BMI - body mass index; NYHA - New York Heart Association; CCS - Canadian Cardiovascular Society; CAD- coronary artery disease; COPD - chronic obstructive pulmonary disease; PAD - peripheral artery disease.

	AS and CAD (N=49)	CAD (N= 29)	P value
Male (%), Female%	27 (55.2%), 22 (44.8%)	16 (55.1%), 13 (44,9%)	0.995
Age (mean; SD)	74.76 ± 12.4	73.34 ± 4.5	0.473
Height (cm)	166.49 ± 9.3	166.24 ± 17.4	0.668
BMI (kg/m2)	28.12 ± 4.3	28.04 ± 3.2	0.840
NYHA class (mean; SD)	2.51 ± 0.8	1.59 ± 1.1	0.001
CCS class (mean; SD)	1.35 ± 1.2	1.72 ± 1.4	0.224
Hypertension (%)	42 (91.3%)	27 (93.1%)	0.780
CAD (%)	35 (71.4%)	21 (72.4%)	0.926
Diabetes (%)	13 (27.1%)	8 (27.6%)	0.962
Dyslipidemia (%)	41 (87.2%)	28 (96.6%)	0.172
Hypothyroidism (%)	9 (19.1%)	3 (10.3%)	0.307
Smoking (%)	7 (14.9%)	12 (41.4%)	0.010
COPD (%)	3 (6.5%)	3 (10.3%)	0.552
PAD (%)	14 (29.8%)	5 (17.2%)	0.220

Table 2. Laboratory characteristics. AS- aortic stenosis; CAD-coronary artery disease; MPV- mean platelet volume; PDW- platelet distribution width; INR- international normalized ratio; APTT- activated partial thromboplastin time; eGFR - estimated glomerular filtration rate; TSH - thyroid-stimulating hormone; LDL - low-density lipoprotein; HDL - high-density lipoprotein.

	AS and CAD (N=49)	CAD (N= 29)	P value
Red blood cell [10^6/uL]	4.32 ± 0.6	4.43 ± 0.5	0.374
Hemoglobin [g/dL)	13.17 ± 1.8	13.32 ± 1.8	0.432
Hematocrit [%]	38.77 ± 5	40 ± 4	0.243
MCV [fL]	87.9 ± 13.3	90.35 ± 3.9	0.294
White blood cell [10^3/uL]	7.72 ± 1.9	7.11 ± 1.6	0.117

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Neutrophils [10³/uL]	5.02 ± 1.8	4.55 ± 1.2	0.278
Lymphocytes [10³/uL]	1.74 ± 0.6	1.81 ± 0.5	0.438
Platelets [10³/uL]	211.92 ± 61.1	217.93 ± 66	0.616
MPV [fL]	10.7 ± 0.8	10.87 ± 1	0.559
PDW [%]	12.56 ± 1.9	13.01 ± 2.3	0.522
INR	1.05 ± 0.1	1.05 ± 0.1	0.923
APPT [sec.]	29.92 ± 4.9	30.05 ± 8.5	0.661
Glucose [mg/dL]	103.71 ± 23.9	103.48 ± 15.1	0.678
Creatinine [mg/dL]	1.13 ± 0.6	0.9 ± 0.1	0.012
eGFR Cockcroft-Gault [mL/min]	64.91 ± 22.2	75.21 ± 15.3	0.009
Total cholesterol [mg/dL]	159.85 ± 40.3	144.41 ± 43.7	0.062
LDL [mg/dL]	84.48 ± 35.6	74.93 ± 39.5	0.203
HDL [mg/dL]	54.54 ± 19.1	49.1 ± 10.7	0.377
Triglycerides [mg/dL]	104.58 ± 29.8	101.93 ± 48.4	0.147

2.2. Echocardiographic Characteristics of AS Group

The echocardiographic characteristic of the study group was as follows: the aortic valve area (AVA) 0.78 ± 0.2 cm² , the maximum transvalvular velocity (Vmax) 4.28 ± 0.6 m/s and the mean pressure gradients (Pmean) 44.82 ± 14.6 mmHg. Among the study group there were 43 patients with severe AS (23 males, mean age: 75.53 ± 11.65 years) and 6 patients with moderate AS (4 males, mean age: 69.17 ± 16.92 years).

The comparative analysis of echocardiographic parameters between the study groups identified several statistically significant differences, reflecting the underlying pathological characteristics associated with AS. Patients in the AS group demonstrated increased interventricular septum (IVS) thickness (14.49 ± 2.7 mm vs. 11.66 ± 2.9 mm; $p < 0.001$) and posterior wall (PW) thickness (11.29 ± 1.6 mm vs. 9.34 ± 1.5 mm; $p < 0.001$) relative to the control group. A comparative analysis of left ventricular ejection fraction (LVEF) revealed no substantial disparities between the two groups. However, the left ventricular global longitudinal strain (LV GLS) was impaired in the AS group compared to the control group (-13.99 ± 3.1% vs. -16.91 ± 3.3%; p = 0.001). The valvulo-arterial impedance (Zva) was notably higher in the AS group (5.5 ± 1.8 vs. 4.47 ± 1.1; $p = 0.003$). Furthermore, the early mitral inflow velocity and the early diastolic mitral annular velocity ratio (E/E′ ratio) was significantly elevated in AS patients (15.88 ± 6.6 vs. 10.43 ± 4.3; $p < 0.001$). The echocardiography characteristics is shown in Table 3.

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Table 3. Echocardiography characteristics. AS- aortic stenosis; CAD-coronary artery disease; LV - left ventricular; EDD – end-diastolic diameter; ESD – end-systolic diameter; EDV - end-diastolic volume; ESV – end-systolic volume; EF – ejection fraction; SVi - stroke volume index; GLS - global longitudinal strain; IVS – interventricular septum thickness; PW – posterior wall thickness; Zva – valvulo-arterial impedance; LA area – left atrium area; E/E' – ratio of early mitral inflow velocity to early diastolic mitral annular velocity.

	AS and CAD (N=49)	CAD (N= 29)	P value
Left ventricular parameters			
LV EDD [mm]	48.2 ± 6.4	50.62 ± 7.4	0.184
LV ESD [mm]	30.41 ± 7.6	30.83 ± 7.2	0.642
IVS [mm]	14.49 ± 2.7	11.66 ± 2.9	0.001
PW [mm]	11.29 ± 1.6	9.34 ± 1.5	0.001
LV EDV [ml]	117.76 ± 40.9	114.76 ± 29.4	0.873
LV ESV [ml]	55.25 ± 36.6	51.38 ± 17	0.465
LV EF [%]	55.1 ± 11.5	55.17 ± 5.2	0.222
LV SVi [ml/m2]	36.31 ± 10.5	32.98 ± 6.9	0.149
LV GLS [%]	-13.99 ± 3.1	-16.91 ± 3.3	0.001
Other parameters			
Zva [mmHg/ml/m²]	5.5 ± 1.8	4.47 ± 1.1	0.003
LA area [cm²]	22.41 ± 3.9	21.93 ± 5	0.608
E/E'	15.88 ± 6.6	10.43 ± 4.3	0.001

2.3. Platelet Aggregometry

A comparison of the platelet aggregation levels measured using the ASPI test revealed no statistically significant differences between AS group and the control group (474.04 ± 66.7 ARU vs. 471.31 ± 56.2ARU; p = 0.822) as well as the ADP test (224.88 ± 46.4 PRU vs. 216.62 ± 29.6 PRU; p=0.394).

2.4. Biomarkers Serum Levels: AS with CAD vs. CAD

The following differences in biomarkers serum levels were observed between the AS and the control group: a significant increase in TM (7.64 ± 3.5 ng/ml vs. 6.28 ± 2.1 ng/ml, p = 0.011) and PF4 (25.16 ; Q1: 8.3; Q3: 29.6 μg/mL vs. 12.85 ; Q1: 5.7; Q3: 14.5 μg/mL, p = 0.021). P-selectin and CD40L levels did not differ between groups. The results are shown in Table 4.

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Table 4. Results of biomarkers and platelet aggregometry. AS- aortic stenosis; CAD-coronary artery disease; TM-thrombomodulin; PF4- platelet factor 4; ASPI – VerifyNow Aspirin test; ARU- aspirin reaction unit; ADP-VerifyNow P2Y12 test; PRU - P2Y12 Reaction Units; Q1—first quartile; Q3—third quartile.

	AS and CAD (N=49)	CAD (N= 29)	P value
TM [ng/ml]	7.64 ± 3.5	6.28 ± 2.1	0.011
PF4 [μg /ml]	25.16 Q1: 8.3; Q3: 29.6	12.85 Q1: 5.7; Q3: 14.5	0.021
P-selectin [ng/ml]	55.83 ± 20.4	54.87 ± 23	0.747
CD40L [ng/ml]	4.59 Q1: 2.2; Q3: 6;	4.85 Q1: 1.8; Q3: 8.2	0.656
ASPI [ARU]	474.04 ± 66.7	471.31 ± 56.2	0.822
ADP [PRU]	224.88 ± 46.4	216.62 ± 29.6	0.394

2.5. Correlation Analysis

Pearson's analysis yielded multiple statistically significant correlations. A negative correlation was identified between ADP-test values expressed in PRUs and hemoglobin (r= -0.38; p= 0.001) as well as hematocrit (r= -0.487; p = 0.001). A negative correlation was also identified between TM and both hemoglobin (r= -0.298, p=0.008) and hematocrit (r= -0.337; p= 0.003). A correlation was observed between P-selectin and CD40L (r=0.362; p= 0.011) concentrations. There were no significant correlations between biochemical markers and severity of AS. The results are shown in Table 5 and Table S1 of the supplementary material.

Table 5. Pearson's correlation analysis. TM- thrombomodulin; PF4- platelet factor 4; PRU - P2Y12 reaction units; r- correlation coefficient; N.S.- not significant.

	Hematocrit	Hemoglobin	CD 40L
P-selectin	r = 0.2659 p = 0.019	r = 0.2471 p = 0.030	r = 0.3617 p = 0.011
PF 4	N.S.	N.S.	N.S.
TM	r = -0.3369 p = 0.003	r = -0.2978 p = 0.008	N.S.
PRU	r = -0.4871 p = 0.001	r = -0.3797 p = 0.001	N.S.

2.6. 12-Month Follow-Up

After a 12-month follow-up period, the following observations were obtained. The incidence of major bleeding (BARC>1) did not demonstrate statistically significant differences between the two groups (4.1% vs. 10.3%; p=0.275).

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3. Discussion

The present study provides new insights into the complex interplay between platelet activation, endothelial dysfunction, inflammation and efficacy of antiplatelet therapy in patients with AS. We observed significantly elevated levels of TM and PF4 in patients with concomitant AS and CAD compared to those with CAD alone. These findings underscore the heightened state of endothelial dysfunction and platelet activation associated with AS, suggesting that valvular pathology contributes independently to the hemostatic and inflammatory profile of those patients. These results support the hypothesis that altered hemodynamics in AS, particularly turbulent flow and increased shear stress, contribute to a prothrombotic and proinflammatory microenvironment within the aortic valve [1,7]. This physical stress can activate platelets, leading to the release of PF4 and other factors implicated in the process of clot formation.

The elevated TM levels observed in AS patients suggest ongoing endothelial damage. TM, an integral membrane protein expressed on endothelial cells, plays a crucial role in the regulation of coagulation and inflammation [31]. Its increased TM presence in the serum may reflect a compensatory response to persistent shear stress and endothelial dysfunction, as previously hypothesized. In AS, increased TM levels are suggestive of an effort to regulate thrombin activity and potentially prevent excessive clotting. The significantly higher TM levels in the AS with CAD group suggest that AS exacerbates endothelial stress beyond that observed in CAD alone. This is consistent with prior studies showing that AS is associated with mechanical stress-induced endothelial disruption, inflammatory signaling, and extracellular matrix remodeling within the aortic valve [1,32–34].

Similarly, PF4 is a chemokine stored in the alpha granules of platelets and released upon activation. Increased PF4 levels indicate ongoing platelet activation and have been associated with thrombo-inflammatory processes in cardiovascular diseases [24,25]. The greater PF4 concentrations in the AS with CAD cohort suggest that AS contributes to a pro-thrombotic state, likely due to altered hemodynamics and shear stress across the stenotic valve, which are known to trigger platelet activation. Elevated levels of PF4 observed in AS may contribute to a prothrombotic state, thereby increasing the risk of thromboembolic events [35].

Interestingly, levels of P-selectin and CD40L, both implicated in platelet-mediated inflammation, did not differ significantly between groups. This may indicate that while endothelial damage and primary platelet activation are pronounced in AS, the secondary inflammatory signaling via these pathways may be less dominant, or more variable, in this particular population. Alternatively, these markers may exhibit a more transient elevation, which was not captured at the time of sampling.

Importantly, in our study we did not find significant difference in platelet aggregation in response to aspirin therapy between AS patients and controls. The aspirin use leaded to adequate platelet inhibition, irrespective of the presence of AS. The analysis revealed that the ARU values were comparable in both groups, with a value less than 550 being observed in both populations. The present findings suggest that excessive platelet activation may occur in a local manner at the site of excessive shear force on the aortic valve and aortic wall [1]. In addition, despite heightened platelet activation, the functional responsiveness to aspirin remains preserved. Consequently, standard antiplatelet therapy may remain effective in attenuating platelet aggregation in this cohort, although the potential contribution of aspirin resistance cannot be entirely excluded and warrants further investigation. The study also assessed baseline platelet function and the degree of platelet inhibition using an ADP assay. This assessment is of limited value because the patients only used aspirin and did not take a P2Y12 inhibitor. In both groups, the PRU values were similar and above 180, suggesting that platelet inhibition was not adequate in both groups (due to the absence of drug). AS exhibited no substantial impact on the aforementioned method of evaluating platelet function.

We also revealed a negative correlation between the extent of platelet activation, as measured by the ADP test and expressed in PRU, and hemoglobin and hematocrit levels. This phenomenon may be attributed to the observation that excessive blood loss and hemolysis can result in excessive

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platelet activation [36]. Hemolysis results in the release of ADP from erythrocytes, which can subsequently activate platelets and enhance platelet aggregation [36–38]. This process may significantly influence the outcomes of ADP-based platelet function tests. Some studies suggest a relationship between hemoglobin and platelet reactivity, potentially indicating that low hematocrit and hemoglobin levels might be associated with increased platelet reactivity [39–41]. If hemolysis is present, ADP tests might show increased platelet aggregation, even if the patient's underlying platelet function is normal. Such false-positive results can lead to misinterpretation of platelet activity and may contribute to inappropriate clinical decisions [42]. Consequently, it is essential to consider the possibility of hemolysis when interpreting ADP test findings, particularly in clinical scenarios where hemolysis is likely or suspected.

The study also included an assessment of the incidence of bleeding (BARC> 1) at 12-month follow-up. Patients diagnosed with AS and CAD did not demonstrate a statistically significant increase in the risk of bleeding complications. Therefore, it can be concluded from this observation that the use of aspirin does not result in a significant increase in the risk of bleeding and can be utilized in this group. Nonetheless, the study was conducted on a relatively small group of participants. It is therefore necessary to conduct additional studies with a larger number of participants in order to confirm this observation.

These findings highlight the multifaceted role of platelet-endothelial interactions in AS. While traditional structural valvular degeneration has long been the primary focus in AS research, our data suggest that systemic vascular and hemostatic alterations may significantly contribute to disease progression and prognosis. The identification of elevated TM and PF4 levels as potential biomarkers of subclinical vascular injury could have prognostic and therapeutic implications, particularly in tailoring anti-inflammatory or antithrombotic strategies in selected patients.

Future studies with larger cohorts and longitudinal biomarker assessments are warranted to validate these observations and explore their utility in risk stratification and treatment personalization. Additionally, exploring the impact of alternative antiplatelet or anti-inflammatory regimens may further elucidate the therapeutic potential of targeting platelet-endothelial interactions in AS.

4. Materials and Methods

4.1. Materials

A total of 78 adult patients with stable CAD therapy participated in the study (43 males, average age: 74.23 ± 10.2 years), including 49 consecutive patients with AS (27 males, average age: 74.76 ± 12.4 years) and 29 control subjects (16 males, average age: 73.34 ± 4.5 years).

The participants of the study were patients admitted to a local cardiology center between July 2021 and December 2023. The study group consisted of patients with moderate to severe AS who were using aspirin (75 mg per day) as part of their pharmacotherapy. The control group comprised individuals matched for age and gender who did not suffer from aortic valve disease and primarily exhibited CAD, with aspirin (75 mg per day) administered as well.

Both groups underwent comprehensive screening, with a review of their medical history. The following exclusion factors from the study were identified: congenital heart disease, cancer, autoimmune disorders, infections, pregnancy, hematologic conditions (such as thrombophilia or hemophilia A/B), infective endocarditis, chronic kidney disease (stage IV-V, eGFR <30 ml/min), chronic dialysis, liver dysfunction (elevated hepatic aminotransferases), or those who did not provide informed consent. The baseline characteristics of both groups are summarized in Table 1.

The study adhered to the Declaration of Helsinki and received approval from the local Bioethics Committee. All participants provided written consent before enrollment.

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4.2. Clinical Assessment

Each participant enrolled in the study underwent a comprehensive clinical evaluation that included a detailed classification according to the New York Heart Association (NYHA) and Canadian Cardiovascular Society (CCS) functional scales. A comprehensive medical history was also obtained, with particular attention to the presence of comorbid conditions, including atrial fibrillation, hypertension, coronary artery disease, diabetes mellitus, dyslipidemia, hypothyroidism, tobacco use, peripheral artery disease, and chronic obstructive pulmonary disease. Standardized physical examinations were conducted, incorporating measurements of anthropometric and hemodynamic parameters such as body weight, height, body mass index (BMI), systolic and diastolic blood pressure, and heart rate. Furthermore, each patient underwent a 12-lead electrocardiogram (ECG), transthoracic two-dimensional echocardiography.

In addition, both study groups were subjected to routine laboratory analyses as part of the diagnostic protocol. The evaluations encompassed a comprehensive array of blood count parameters, including red and white blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), neutrophils, lymphocytes, platelet count, mean platelet volume (MPV), and platelet distribution width (PDW). Liver function was assessed through the measurement of alanine aminotransferase (ALT), and metabolic indicators such as glucose were also evaluated. The following variables were measured: creatinine, estimated glomerular filtration rate (eGFR) calculated using the Cockcroft–Gault equation, electrolyte levels (sodium and potassium), thyroid-stimulating hormone (TSH), and lipid profile components such as triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

4.3. Transthoracic Echocardiography

Transthoracic echocardiographic evaluations were performed using a 2.5-MHz transducer and included two-dimensional (2D), M-mode, and Doppler imaging modalities. The echocardiographic assessments were conducted by a cardiologist with extensive experience in the field, in accordance with the guidelines established by the European Association of Cardiovascular Imaging (EACVI) [43]. Furthermore, offline analysis employing two-dimensional speckle-tracking echocardiography was utilized to ascertain left ventricular global longitudinal strain (LV GLS). The echocardiographic protocol encompassed the measurement of several key structural and functional cardiac parameters, including: The following variables were measured: the aortic valve area (AVA), the peak transvalvular velocity (Vmax), the peak (Pmax), and the mean (Pmean) pressure gradients; the left ventricular end-diastolic and end-systolic diameters (LV EDD, LV ESD); the end-diastolic and end-systolic volumes (LV EDV, LV ESV); the left ventricular outflow tract (LVOT) diameter; the left ventricular ejection fraction (LVEF); the stroke volume index (SVi); and the left atrial area (LA area). Furthermore, myocardial wall thicknesses of the interventricular septum (IVS) and the posterior wall (PW) were recorded. Diastolic function was assessed by measuring early mitral inflow velocity (E wave), early diastolic mitral annular velocity (E′), and the E/E′ ratio. Furthermore, the valvulo-arterial impedance (Zva) was calculated.

4.4. Biomarkers – Fluorescent Bead-Based Luminex Assays

Venous blood samples (10 mL) were obtained from the antecubital vein of all study participants. Serum specimens designated for analysis were aliquoted into polypropylene tubes and stored at −80°C until further processing. The quantification of biomarkers was conducted using a magnetic Luminex assay, with the procedure performed in accordance with the manufacturer's guidelines. Prior to analysis, serum samples were diluted as specified by the protocol and immediately subjected to assay procedures. The concentrations of TM, P-selectin, PF4, and CD40L were determined using multiplex bead-based immunoassays (Luminex technology) on the Bio-Plex 200 suspension array system (R&D Systems, Minneapolis, USA), strictly following the manufacturer's instructions. The acquisition of data was executed through the utilization of a Bio-Plex 200 instrument, a device that

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has been validated and calibrated (Bio-Rad Laboratories, Watford, UK). The subsequent processing of results was facilitated by employing Bio-Plex Manager 6.0 software, which is also manufactured by Bio-Rad Laboratories (Watford, UK). For the purpose of assay calibration, the detection target was configured to capture 50 beads per analyte region. This was achieved by employing a low RP1 target for CAL2 calibration. The doublet discrimination gates were set between 5,000 and 25,000, in accordance with the recommended settings for the Bio-Plex platform. The primary readout metric—median fluorescence intensity (MFI)—was recorded and utilized for subsequent quantitative analysis.

4.5. Platelet Function Tests - Optical Aggregometry

The platelet reactivity following aspirin treatment was measured using the VerifyNow Aspirin assay, while efficacy of P2Y12 was measured using the VerifyNow-P2Y12 assay. Tests were was obtained following the previously published method [44,45]. Blood samples were obtained from all patients and collected in 3.2% citrate Vacuette tubes (Greiner Bio-One, Kremsmünster, Austria). The blood samples were stored at ambient temperature. Tests were carried out in strict accordance with the manufacturers' guidelines. The test is based on turbidimetric detection, where the extent of light transmittance is proportional to the degree of platelet-induced aggregation of fibrinogen-coated beads. In the VerifyNow Aspirin Assay (ASPI test), arachidonic acid functions as the platelet agonist. Under normal physiological conditions, arachidonic acid is converted via the cyclooxygenase-1 (COX-1) enzyme pathway into thromboxane A2 (TxA2), a potent stimulator of platelet aggregation. Aspirin exerts its antiplatelet effects by irreversibly inhibiting COX-1, thereby reducing the formation of thromboxane A2 and ultimately preventing the activation of the GPIIb/IIIa receptor, which is essential for platelet aggregation. An Aspirin Reaction Unit (ARU) value of 550 or higher is indicative of normal platelet function and the absence of significant aspirin-induced inhibition, suggesting that the patient is either non-aspirin users or exhibits aspirin resistance. Conversely, an ARU value below 550 indicates platelet dysfunction, consistent with the presence and efficacy of aspirin. The VerifyNow-P2Y12 assay (ADP test) is a rapid diagnostic test that employs adenosine diphosphate (ADP) to induce platelet activation in the presence of prostaglandin E1 (PGE1), which serves to suppress signaling via the secondary ADP receptor, P2Y12. This design enhances the assay's sensitivity and specificity for detecting the functional activity of the P2Y12 receptor and the pharmacodynamic effects of P2Y12 receptor antagonists. A result that exceeds 180 P2Y12 Reaction Units (PRU) indicates the absence of P2Y12 inhibitor activity, whereas a PRU value of 180 or below reflects a reduced platelet reactivity consistent with the presence of a P2Y12 receptor inhibitor.

4.6. 12-Month Follow-Up

Participants were monitored over a 12-month period through scheduled outpatient clinic visits or, in selected cases, via telephone consultations conducted in the outpatient setting. During the follow-up period, the occurrence of major bleeding (BARC > 1) was systematically documented.

4.7. Statistical analysis

All statistical analyses were conducted using Statistica software, version 13.3 (StatSoft, Krakow, Poland). Continuous variables were expressed as means ± standard deviation (SD) for normally distributed data, or as medians with interquartile ranges (1st and 3rd quartiles) for non-normally distributed data. Categorical variables were presented as absolute counts and corresponding percentages. The Shapiro–Wilk test was used to assess the normality of data distribution. For comparisons between groups, the Student's t-test was used for normally distributed continuous variables, while the U Mann–Whitney test was applied to those not meeting normality assumptions. Differences in categorical variables were assessed using the chi-squared test. Person's coefficient was used to evaluate associations between biomarker concentrations and other continuous or ordinal variables. Variables exhibiting a p-value < 0.1 in univariate regression analysis were entered into the

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multivariate regression model. A two-sided p-value < 0.05 was considered indicative of statistical significance.

5. Conclusions

The study examined the relationship between AS, endothelial dysfunction, and platelet activation by assessing specific serum biomarkers and platelet aggregation tests. We found that the coexistence of AS in patients with CAD was associated with elevated levels of biomarkers indicative of endothelial damage and platelet activation. On the other hand, the aggregation test representing the efficacy of antiplatelet treatment was comparable between the groups and no differences of bleeding complications were observed. The findings highlight the role of endothelial and platelet interactions in patients with CAD and AS and suggest a safety of the antiplatelet therapy in this patient population.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Pearson's correlation analysis

Author Contributions: Conceptualization, Paweł Bańka and Katarzyna Mizia-Stec; Data curation, Paweł Bańka, Maciej Wybraniec and Katarzyna Mizia-Stec; Formal analysis, Paweł Bańka, Maciej Wybraniec and Katarzyna Mizia-Stec; Funding acquisition, Katarzyna Mizia-Stec; Investigation, Paweł Bańka, Kinga Czepczor, Maciej Podolski, Agnieszka Kosowska, Wojciech Garczorz, Tomasz Francuz, Maciej Wybraniec and Katarzyna Mizia-Stec; Methodology, Paweł Bańka and Katarzyna Mizia-Stec; Project administration, Paweł Bańka and Katarzyna Mizia-Stec; Resources, Katarzyna Mizia-Stec; Software, Paweł Bańka, Maciej Wybraniec and Katarzyna Mizia-Stec; Supervision, Maciej Wybraniec and Katarzyna Mizia-Stec; Validation, Paweł Bańka, Agnieszka Kosowska, Wojciech Garczorz, Tomasz Francuz and Katarzyna Mizia-Stec; Visualization, Paweł Bańka and Katarzyna Mizia-Stec; Writing – original draft, Paweł Bańka and Katarzyna Mizia-Stec; Writing – review & editing, Paweł Bańka and Katarzyna Mizia-Stec.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy, intellectual property rights and ethical reasons.

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Abbreviations

The following abbreviations are used in this manuscript:

AS	aortic stenosis
AVA	aortic valve area
BMI	body mass index
CAD	coronary artery disease
CCS	Canadian Cardiovascular Society
CD	cluster of differentiation

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COPD	chronic obstructive pulmonary disease
E/E'	ratio of early mitral inflow velocity to early diastolic mitral annular velocity
EDD	end-diastolic diameter
EDV	end-diastolic volume
EF	ejection fraction
ESD	end-systolic diameter
ESV	end-systolic volume
eGFR	estimated glomerular filtration rate
GLS	global longitudinal strain
HDL	high-density lipoprotein
IVS	interventricular septum thickness
LA area	left atrium area
LDL	low-density lipoprotein
LV	left ventricular
MACCE	major adverse cardiovascular and cerebrovascular event
MCV	mean corpuscular volume
MPV	mean platelet volume
NYHA	New York Heart Association
PAD	peripheral artery disease
PDW	platelet distribution width
PF4	platelet factor 4
Pmax	maximum pressure gradient
Pmean	mean pressure gradient
PW	posterior wall thickness
TM	thrombomodulin
Q1	first quartile
Q3	third quartile
SVi	stroke volume index
TM	thrombomodulin
Vmax	maximum velocity
VICs	valvular interstitial cells
Zva	valvulo-arterial impedance

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