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Journal of Cardiology 65 (2015) 501–507

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Exercise stress testing enhances blood coagulation and impairs fibrinolysis in asymptomatic aortic valve stenosis

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ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 20 July 2014
Accepted 30 July 2014
Available online 26 August 2014

Keywords:
Aortic valve stenosis
Exercise
Thrombin potential
Fibrinolysis

ABSTRACT

Background: Increased thrombin formation and fibrin deposition on the valves were observed in patients with severe aortic valve stenosis (AS). Exercise enhances blood coagulation and fibrinolysis in healthy subjects, but its haemostatic effects in AS are unknown. We sought to investigate how stress echocardiography alters blood coagulation and fibrinolysis in AS patients free of significant atherosclerotic vascular disease.
Methods: We studied 32 consecutive asymptomatic moderate-to-severe AS patients and 32 age- and sex-matched controls. We measured peak thrombin generated using calibrated automated thrombogram, clot lysis time (CLT), and fibrinolytic markers at four time points, i.e. at rest, at peak exercise, and 1 h and 24 h after a symptom-limited exercise test.
Results: We observed that peak thrombin generated rose at peak exercise to 25% higher value in the patients than in controls ($p < 0.001$) and reached its highest level 24 h from exercise in AS patients while it decreased post-exercise in controls. Baseline CLT was 13% longer in AS patients ($p = 0.006$) and associated with thrombin activatable fibrinolysis inhibitor (TAFI) activity ($r = 0.69$, $p < 0.001$), antiplasmin ($r = 0.47$, $p = 0.007$), and plasminogen ($r = -0.55$, $p = 0.001$). In AS, CLT remained unaltered at peak exercise, while it decreased in controls, yielding the intergroup difference of 28% ($p < 0.001$). Twenty-four hours after exercise CLT became 15% longer in AS patients ($p < 0.001$). This hypofibrinolytic effect followed a similar pattern observed for TAFI activity.
Conclusions: Asymptomatic moderate-to-severe AS patients respond to exercise with more pronounced and prolonged increase in thrombin generation, together with impaired fibrinolysis as compared to controls. Repeated episodes of exercise-induced prothrombotic state in AS might contribute to the progression of this disease.
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Introduction

Aortic valve stenosis (AS) is the most common valvular heart disease in adults, which could be asymptomatic for a long time [1]. Current evidence indicates that AS is an active process with lipoprotein infiltration, chronic inflammation, extracellular matrix remodeling, angiogenesis, and calcium deposition [2,3], which is to some extent similar to atherosclerosis, in which enhanced blood coagulation is involved in its progression and complications [4].

There have been reports suggesting that AS is also associated with enhanced blood clotting. Dimitrow et al. [5] have shown that there is a prothrombotic state, characterized by increased thrombin formation and platelet activation associated with maximal transvalvular gradient in patients with severe AS. Altered hemodynamic properties of blood flow with the occurrence of post-stenotic turbulence in AS can facilitate the activation of the coagulation cascade with the resultant thrombin generation [5]. Microthrombi on the aortic valve have been shown in AS patients [6]. It has been demonstrated that fibrin accumulation occurs within and on the surface of diseased valves in AS patients, and it is associated with thrombin generation and fibrin turnover in circulating blood [7]. Moreover, tissue factor (TF) is abundantly expressed in human stenotic aortic valves in association with thrombin formation in circulating blood [8,9]. The loss of high

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http://dx.doi.org/10.1016/j.jjcc.2014.07.019
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molecular weight multimers of von Willebrand factor (vWF) owing to high shear stress lesion and vWF-mediated platelet dysfunction can also be observed in patients with severe AS and predispose to bleeding [10]. Recently, we have reported that hypofibrinolysis is more common in AS patients than in controls [11].

It is known that exercise can induce a prothrombotic state [12]. Beneficial effects of physical activity on the risk of coronary artery disease (CAD) and cardiovascular mortality may result at least in part from increased fibrinolysis following exercise [12,13]. To our knowledge, there have been no studies on the effect of exercise on blood coagulation and fibrinolysis in AS patients. The present study was performed to evaluate potential differences in the hemostatic response to exercise test in AS patients vs. controls.

Materials and methods

Patients

Thirty-three consecutive adult patients with asymptomatic moderate-to-severe AS [defined as transvalvular maximal velocity (V_{max}) ≥3 m/s] were recruited from March 2011 to June 2012.

The exclusion criteria were: history of angina, dizziness, syncope, another cardiac valve disease of more than a moderate degree, left ventricular (LV) ejection fraction (EF) <50%, history of or current atrial fibrillation, hyper- or hypothyroidism, diabetes treated with insulin, renal or hepatic dysfunction, lung disease, oral anticoagulant therapy, use of thienopyridine or nonsteroidal anti-inflammatory drugs other than aspirin, known cancer, bleeding tendency, autoimmune disorders, a history of myocardial infarction, stroke, or venous thromboembolism. Patients who were not able to perform exercise testing were also excluded from the study. Sex- and age-matched individuals recruited from the families of hospital personnel served as controls. The study protocol was approved by the University Bioethical Committee, and each patient provided written, informed consent to participate in the study.

To evaluate the extent of atherosclerotic vascular disease, which coexists in about 50% of AS patients [14], we measured intima–media thickness (IMT) in both right and left common carotid artery in accordance with the Mannheim IMT consensus [15]. The ankle brachial pressure index (ABI) was measured using an arterial pressure sphygmomanometer and a continuous wave Doppler ultrasound blood flow detector and values of 0.9–1.15 were considered normal. Hypertension was diagnosed based on a history of hypertension or antihypertensive treatment. Hyperlipidemia was diagnosed based on medical records, statin therapy, or total cholesterol of ≥5.0 mmol/L.

Echocardiography

Transthoracic echocardiography was performed in all enrolled subjects using Philips iE33 (Philips Electronics, Andover, MA, USA). LV volumes and EF were measured by the biplane Simpson's method. The aortic valve area (AVA) was calculated using the standard continuity equation. V_{max}, peak pressure gradient (PPG), and mean pressure gradient (MPG) were calculated using the modified Bernoulli equation.

A symptom-limited exercise stress echocardiography was performed on a bicycle ergometer (Ergoline, Bitz, Germany) in a semisupine position with a continuous echocardiographic examination by an experienced cardiologist. After 3 min of the initial workload of 25 W, the workload was increased every 3 min by 25 W [16]. Electrocardiogram was monitored and blood pressure was measured every 3 min during exercise. Exercise was stopped in case of typical chest pain, breathlessness, dizziness, muscular exhaustion, hypotension, ventricular arrhythmia, when age-related maximum heart rate was reached, or on the patient's demand. The test was performed at rest and peak exercise.

Laboratory tests

Fasting blood samples were drawn from the antecubital vein between 07.00 h and 10.00 h. Fibrinogen was measured by the von Clauss method. High-sensitivity C-reactive protein was determined using immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). Blood samples to determine hemostatic parameters were drawn four times: at rest, at peak exercise, and 1 h and 24 h after exercise and then centrifuged at 2500 × *g* at 20 °C for 10 min and stored at −80 °C until analysis. Technicians were blinded to the origin of the samples.

Thrombin generation

Measurement of the thrombin endogenous potential was performed using calibrated automated thrombography (Thrombinoscope BV, Maastricht, Netherlands) according to the manufacturer's instructions in the 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland). Eighty microliters of platelet-poor plasma was diluted with 20 μL of the reagent containing 5 pmol/L recombinant TF, 4 μM phosphatidylserine/phosphatidylcholine/phosphatidylethanolamine vesicle, and 20 μL of FluCa solution (HEPES, pH 7.35, 100 nmol/L $CaCl_2$, 60 mg/mL bovine albumin, and 2.5 mmol/L Z-Gly-Gly-Arg-AMC). Samples were analyzed in duplicate; intra-assay variability was 5.7%. We analyzed the maximum concentration of thrombin generated.

Endothelial cell activation markers

Plasma soluble thrombomodulin (TM) (Diagnostica Stago, Asniéres, France) and tissue plasminogen activator (tPA) antigen (Hyphen BioMed, Neuville-Sur-Oise, France) were determined by enzyme-linked immunosorbent assays (ELISA).

Fibrinolysis

Measurement of thrombin activatable fibrinolysis inhibitor (TAFI) antigen was performed with an ELISA (Chromogenix, Lexington, MA, USA). The activity levels of plasminogen activator inhibitor-1 (PAI-1) and TAFI were measured using a chromogenic assay (Chromolize PAI-1, Trinity Biotech, Bray, County Wicklow, Ireland; and ACTICHROME® Plasma TAFI Activity Kit, American Diagnostica, Greenwich, CT, USA, respectively).

PAI-1 antigen was determined in citrated plasma using commercially available ELISAs according to the manufacturer's instructions (American Diagnostica).

Plasminogen and α2-antiplasmin (α2-AP) were measured by chromogenic assays (Diagnostica Stago).

Plasma D-dimer was determined by immunoturbidimetry (Innovance D-dimer, Siemens, Erlangen, Germany). Intra-assay and inter-assay coefficients of variation were <8%.

Clot lysis time (CLT) was measured as previously described [17] with some modifications. Briefly, citrated plasma was mixed with Tris buffer (1:1) containing 15 mmol/L calcium chloride, 0.6 pmol/L human TF (Innovin, Siemens, Marburg, Germany), 12 μmol/L phospholipid vesicles (Avanti Polar Lipids, Alabaster, AL, USA), and 60 ng/mL recombinant tPA (Boerhinger Ingelheim, Ingelheim, Germany). Turbidity was measured at 405 nm at 37 °C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents the clot formation, to the midpoint of the maximum-turbid-to-clear transition, representing the lysis of the clot. Intra-assay and inter-assay coefficients of variation were 6.0 and 7.4%, respectively.

Statistical analysis

Statistical analysis was performed using STATISTICA 10 PL software package (StatSoft, Tulsa, OK, USA). Values are presented as a mean ± standard deviation or median or otherwise stated. The Shapiro–Wilk test was performed to determine normal distribution of the variables. The Student's t test was used to determine differences among normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Serial tests were analyzed using Freidman ranks analysis of variance. A linear Pearson correlation was used to assess correlations among variables. A p-value <0.05 was considered statistically significant.

Results

Subject characteristics

Thirty-two AS patients and 32 controls were included in the final analysis (Table 1) due to the fact that one subject from each group was excluded since elevated blood white cell count and hypothyroidism were observed in the respective subjects. Slightly lower hematocrit, hemoglobin, platelet count, and more common hypercholesterolemia were observed in AS patients, while other routine laboratory parameters were similar in both groups (Table 1).

Echocardiography parameters

The indices of the AS severity were: V_{max} = 3.8 ± 0.59 m/s; PPG = 59.4 ± 19.8 mmHg; MPG = 35.4 ± 14.1 mmHg; AVA = 1.08 ± 0.23 cm^2; AVA indexed to body surface area (AVA/BSA) = 0.58 ± 0.1 cm^2/m^2. Bicuspid aortic valves were found in 12 (37.5%) AS patients.

Table 1
Baseline characteristics.

	AS patients (n = 32)	Controls (n = 32)	p
Males, n (%)	15 (46.8)	17 (53.1)	0.62
Age (years)	64 (23–82)	63.5 (33–83)	0.81
Body mass index (kg/m^2)	30 (21–37)	28 (21–39)	0.19
Current smoking, n (%)	2 (6.2)	2 (6.2)	1.0
Hypertension, n (%)	26 (81.2)	27 (84.3)	0.74
Diabetes, n (%)	7 (21.8)	4 (12.5)	0.32
Hypercholesterolemia, n (%)	12 (37.5)	2 (6.2)	0.02
Laboratory investigations			
Hemoglobin (g/dL)	13.5 (11.5–15.9)	14.6 (12.2–16.9)	0.003
Hematocrit (%)	40.7 ± 2.8	42.7 ± 2.7	0.002
Platelets (10^3/μL)	202 (127–356)	225 (142–325)	0.04
Fibrinogen (g/L)	3.26 ± 0.7	3.04 ± 0.5	0.21
Total cholesterol (mM)	5.03 ± 1.04	5.26 ± 1.2	0.41
LDL cholesterol (mM)	3.04 ± 0.8	3.33 ± 1.0	0.24
HDL cholesterol (mM)	1.48 ± 0.3	1.48 ± 0.4	0.92
Triglycerides (mM)	1.15 (0.4–4.2)	1.15 (0.4–2.3)	0.50
Glucose (mM)	5.85 ± 1.1	5.38 ± 0.5	0.30
Creatinine (μM)	73.8 ± 12.4	77.3 ± 13.9	0.28
C-reactive protein (mg/L)	1.23 (0.3–10.4)	1.6 (0.2–8.0)	0.61
Treatment			
Aspirin, n (%)	18 (56.2)	10 (31.2)	0.044
Statins, n (%)	23 (71.8)	20 (62.5)	0.42
β-Blockers, n (%)	19 (59.3)	12 (37.5)	0.08
ACEI, n (%)	12 (37.5)	15 (46.8)	0.45
Vascular ultrasound examination			
IMT (mm)	0.70 ± 0.20	0.71 ± 0.15	0.41
ABI	1.00 ± 0.11	1.05 ± 0.14	0.08

Data are median (interquartile range) or mean ± SD, unless otherwise stated. p-Value was measured using Student's t-test when variables were normally distributed or by the Mann–Whitney U test for non-normally distributed variables. AS, aortic valve stenosis; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ACEI, angiotensin-converting enzyme inhibitor; IMT, intima–media thickness; ABI, ankle brachial pressure index.

The duration of stress test was shorter in AS group (p = 0.008) and maximum workload was lower (p = 0.002) and reached 81.3 ± 21.1 W. During stress test seven (21.8%) patients had dyspnea and three (9.3%) had angina without echocardiographic signs of ischemia in AS patients, while in the control group five patients had dyspnea (15.6%). In the AS group at maximum workload PPG rose to 75.3 ± 26.1 mmHg, MPG to 45.3 ± 15.9 mmHg, and EF to 73 ± 6.5% (all $p < 0.05$). There was no intergroup difference in post-exercise increase in EF.

Thrombin generation

As shown in Fig. 1A and Table 2, peak thrombin generated during peak workload in AS group increased by 25% ($p < 0.001$) and was higher than in controls ($p < 0.001$). Interestingly, peak thrombin generated in AS patients reached its peak 24 h after exercise, while it followed a different pattern in controls, i.e. it decreased reaching its nadir 24 h post-exercise ($p < 0.001$). There was a positive correlation between peak thrombin generated at baseline and left atrium volume (r = 0.42, p = 0.014), but not with other echocardiographic parameters at any time point (data not shown).

Fibrinolysis

As shown in Fig. 1B and Table 2, baseline CLT was 13% longer among AS patients (p = 0.006) compared with controls. CLT remained unaltered at peak exercise in AS group and it decreased in controls (intergroup difference of 28%, $p < 0.001$). One hour after exercise test CLT decreased in AS patients to a level similar to the control group, and then increased 24 h later, being 15% higher than in controls ($p < 0.001$). Exercise showed no effect on D-dimer in either group (Table 2).

TAFI antigen remained unaltered during stress test in the AS group. The only intergroup difference was observed at peak exercise where TAFI antigen was slightly higher in the patients (p = 0.015) (Table 2). In controls TAFI antigen was reduced at peak exercise (p = 0.005) and 1 h after exercise compared to the baseline value (p = 0.030). Interestingly, at all four time points, TAFI activity was higher in AS group than in controls (all $p < 0.001$, Fig. 1C). In AS patients, TAFI activity remained unaltered at peak exercise, but then after a decrease after 1 h it rose above the baseline value (p = 0.003). In controls, TAFI activity was reduced during the test and after its completion compared to the baseline (all $p < 0.01$, Fig. 1C). Of note there was a weak association between TAFI antigen and V_{max} (r = 0.36, p = 0.042).

Plasminogen was similar at baseline in both groups, but then it was higher in AS group (all $p < 0.05$, respectively) (Table 2). There were no differences in PAI-1 antigen between the two groups at any time point. Although α_2-AP remained unaltered during exercise test in the AS group, it was higher than in the controls at baseline (p = 0.024), similar at peak exercise, and lower at 1 and 24 h post-exercise (both $p < 0.05$, Table 2). Importantly, in AS patients CLT at baseline, but not at the three other time points, correlated with TAFI activity (r = 0.69, $p < 0.001$), α_2-AP (r = 0.47, p = 0.007), and plasminogen (r = −0.55, p = 0.001). In controls, CLT correlated with TAFI activity at all time points (at rest: r = 0.65, $p < 0.001$; at peak exercise: r = 0.54, p = 0.001; 1 h post-exercise: r = 0.42, p = 0.015; 24 h post-exercise: r = 0.50, p = 0.003).

Other parameters

Soluble TM in both groups at baseline was similar, but it was higher than in the control group both at peak exercise ($p < 0.001$), 1 h ($p < 0.001$), and 24 h after exercise (p = 0.034) (Fig. 1D and Table 2). Pre-exercise CLT correlated with TM in the AS group

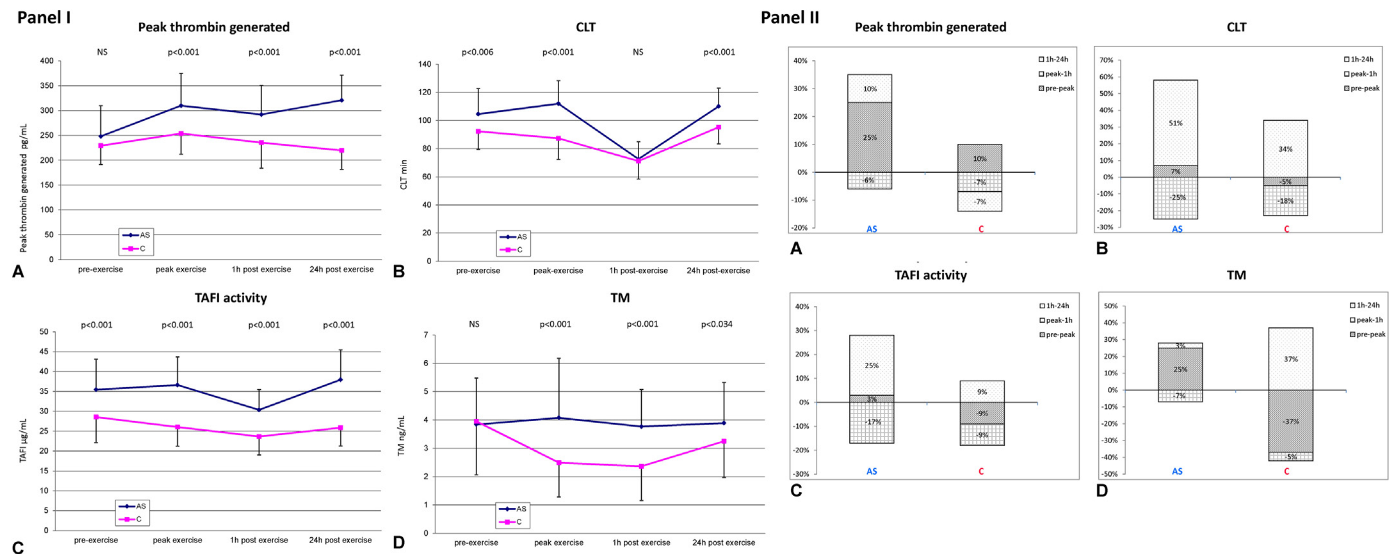

Fig. 1. Exercise-induced changes during symptom-limited exercise on a bicycle ergometer in 32 asymptomatic aortic valve stenosis patients (AS) and 32 well-matched controls (C) in: (A) peak thrombin generated; (B) clot lysis time (CLT); (C) thrombin activatable fibrinolysis inhibitor activity (TAFI); (D) thrombomodulin (TM). Panel I shows absolute values (data are presented as a mean ± SD); Panel II shows percentage changes of the parameters measured in specific time periods: from pre-exercise to peak exercise; from peak exercise to 1 h post-exercise, and from 1 h to 24 h post-exercise (baseline – 0% represents their initial, pre-exercise value).

Table 2
Markers of blood coagulation and fibrinolysis at baseline, peak exercise, and 1 and 24 h after exercise.

	Time point	AS patients (*n* = 32)	Controls (*n* = 32)	*p*
Peak thrombin generated (pg/mL)	Pre-exercise	247 ± 62.0	229 ± 37.7	0.26
	Peak exercise	309 ± 65.1	253 ± 41.9	<0.001
	1 h post-exercise	291 ± 58.6	235 ± 51.2	<0.001
	24 h post-exercise	320 ± 50.9	219 ± 38.2	<0.001
CLT (min)	Pre-exercise	104 ± 18.2	92 ± 12.9	0.006
	Peak exercise	112 ± 16.4	87 ± 15.0	<0.001
	1 h post-exercise	72 ± 12.2	71 ± 12.7	0.47
	24 h post-exercise	110 ± 13.0	95 ± 11.8	<0.001
TAFI activity (μg/mL)	Pre-exercise	35 ± 7.7	28.6 ± 6.4	<0.001
	Peak exercise	36 ± 7.1	26 ± 4.8	<0.001
	1 h post-exercise	30 ± 5.1	23 ± 4.6	<0.001
	24 h post-exercise	37 ± 7.5	25 ± 4.6	<0.001
TAFI antigen (%)	Pre-exercise	103 ± 10.4	105 ± 10.3	0.38
	Peak exercise	106 ± 12.8	99 ± 8.9	0.015
	1 h post-exercise	105 ± 12.2	100 ± 9.6	0.08
	24 h post-exercise	106 ± 11.2	103 ± 10.1	0.23
PAI-1 (ng/mL)	Pre-exercise	51 ± 25.2	55 ± 19.8	0.64
	Peak exercise	51 ± 21.1	57 ± 16.2	0.22
	1 h post-exercise	48 ± 19.3	46 ± 17.6	0.44
	24 h post-exercise	47 ± 19.8	47 ± 18.1	0.92
Plasminogen (%)	Pre-exercise	101 ± 12.3	99 ± 10.8	0.69
	Peak exercise	97 ± 15.6	88 ± 10.2	0.023
	1 h post-exercise	110 ± 13.3	91 ± 14.9	<0.001
	24 h post-exercise	106 ± 27.3	90 ± 15.2	0.019
α_2-AP (%)	Pre-exercise	106 ± 12.0	99 ± 10.4	0.024
	Peak exercise	104 ± 15.8	108 ± 11.6	0.10
	1 h post-exercise	99 ± 15.6	108 ± 11.1	0.011
	24 h post-exercise	99 ± 14.0	108 ± 11.7	0.008
tPA antigen (ng/mL)	Pre-exercise	4.8 ± 1.8	4.3 ± 1.2	0.39
	Peak exercise	5.1 ± 2.3	5.6 ± 2.1	0.37
	1 h post-exercise	3.7 ± 1.4	3.6 ± 1.2	0.96
	24 h post-exercise	3.7 ± 1.6	3.4 ± 1.1	0.56
TM (ng/mL)	Pre-exercise	3.8 ± 1.6	3.9 ± 1.8	0.83
	Peak exercise	4.0 ± 2.1	2.4 ± 1.2	<0.001
	1 h post-exercise	3.7 ± 1.3	2.3 ± 1.2	<0.001
	24 h post-exercise	3.8 ± 1.4	3.2 ± 1.2	0.034
D-Dimer (ng/mL)	Pre-exercise	432 ± 309	441 ± 235	0.51
	Peak exercise	619 ± 629	477 ± 300	0.84
	1 h post-exercise	491 ± 425	543 ± 393	0.51
	24 h post-exercise	585 ± 650	549 ± 520	0.92

Data are given as mean ± SD. *p*-Value was determined using Student's *t*-test.
AS, aortic valve stenosis; CLT, clot lysis time; TAFI, thrombin activatable fibrinolysis inhibitor; PAI-1, plasminogen activator inhibitor-1; α_2-AP, α_2-antiplasmin; tPA, tissue plasminogen activator; TM, thrombomodulin.

($r = 0.55$, $p = 0.001$). There were no differences in tPA antigen between the AS and control groups (data not shown).

Subgroup analysis

Subgroups of AS patients were identified based on: (1) stroke volume indexed to body surface area <35 mL/m^2 and ≥ 35 mL/m^2 (15 vs 17 patients); (2) exercise-induced elevation of MPG >20 mmHg and ≤20 mmHg (27 vs 5 patients); (3) exercise-induced increase in LVEF ≥10% and <10% (21 vs 11 patients). The only significant difference was observed in the peak thrombin generated at 24 h post-exercise (330.2 ± 48.1 pg/mL in the subgroup with MPG elevation ≤20 mmHg vs 268.6 ± 38.1 pg/mL in the subgroup with MPG elevation >20 mmHg, $p = 0.010$).

Discussion

The study is the first to demonstrate that during exercise stress testing moderate-to-severe AS patients show a specific pattern of prothrombotic changes in coagulation and fibrinolysis markers, which is different from that observed in age- and sex-matched controls. Our control group does not consist of healthy subjects, however this sample is representative of the population in this age range. Even in patients with a normal stress test, we observed exercise-induced greater increase in maximum concentration of thrombin generated combined with lower fibrinolysis and those alterations were detectable even 24 h after the test. Of note, those alterations cannot be attributable to significant atherosclerotic vascular disease. Our findings could suggest that unlike in individuals free of AS and/or CAD, physical exercise in asymptomatic AS patients can produce unfavorable effects, which might predispose to faster progression of AS as suggested recently [11]. Our observations apply to patients with moderate-to-severe AS, and cannot possibly be extended to the early stages of this disease.

Thrombin generation

There have been few studies on blood coagulation in AS that reported enhanced thrombin generation using a different methodology [5,18]. Most likely, at rest increased thrombin formation can be detected in symptomatic patients with severe AS and/or with concomitant CAD. Furthermore, we measured the peak thrombin generated, a reliable marker of a prothrombotic state [19], which has not been tested in AS until now. We observed increased peak thrombin generated, not only at peak exercise but even 24 h after exercise in AS patients, while in controls there was a modest increase in the peak thrombin generated at peak exercise, and its decrease 24 h post-exercise. These results in AS patients are different from those observed in healthy young male subjects in whom no changes in endogenous thrombin potential during exercise were observed [20]. Clear differences in the profile of thrombin potential during exercise in AS patients and the controls suggest that regulatory mechanisms of blood coagulation leading to a quick return to baseline values after the exercise test are impaired in AS. The mechanisms behind this observation are unknown and probably several mechanisms might be implicated. It has been postulated that AS and atherosclerosis are similar diseases. Despite a different location, the main interface appears to be the vascular endothelium. Flow turbulence at the supravalvular region in AS patients, increasing during exercise, can cause disruption of the endothelium followed by a local proinflammatory, prothrombotic, and procoagulant state. Furthermore, there is evidence that reduced blood volume may activate thrombin generation [21]. The stroke volume is decreased in AS patients. Higher heart rate during exercise may compensate lower cardiac output among healthy individuals, whereas in AS cardiac output is lower at an increased heart rate due to the wall stiffness, resulting in the diastolic function impairment. Reduced stroke volume, followed by reduced blood volume, might contribute to prothrombotic alterations during exercise in AS patients. It has been also suggested that hemolysis of red blood cells enhances blood coagulation [22]. Intravascular hemolysis related to AS has been reported in a few case reports regarding AS patients [23,24]. However, in a randomly selected subgroup of our AS patients we failed to observe any laboratory indices of hemolysis (Kolasa-Trela, unpublished data). It has been established that thrombin itself can cause endothelial cell perturbation [4], thus hypercoagulability observed in AS may cause further destruction of the valve leaflets. It remains to be established why natural anticoagulant systems, including protein C and antithrombin, cannot quickly shut down the burst of exercise-induced thrombin generation in asymptomatic moderate-to-severe AS.

Fibrinolysis

We observed impaired fibrinolysis in AS patients prior to the exercise test, reflected by prolonged CLT, which is in accordance with a recent study by Natorska et al. [11]. A novel finding is that exercise-induced acceleration of fibrinolysis in AS patients is delayed and transitory as compared to the alterations observed in controls. Interestingly, while CLT shortened in controls at peak exercise, it remained on the same level among AS individuals. We found that shortening of CLT in AS was delayed (1 h post-exercise) and transitory (it was prolonged again 24 h after exercise). The observed fibrinolytic plasma profile in AS cannot be explained by the diurnal variation of fibrinolytic activity because of intergroup differences. A different pattern of change in fibrinolytic potential during exercise in AS also varied from that observed in patients with hypertension, in whom increased coagulation parameters were accompanied by enhanced fibrinolysis [25]. Mechanisms of this observation could be related to TAFI which represents a link between the blood coagulation and fibrinolysis. Unexpectedly, we found that the initial TAFI activity was higher in AS and positively associated with CLT. One of the possible explanations of elevated pre-exercise TAFI activity levels are observations that TAFI has an anti-inflammatory effect through inactivation of anaphylatoxin C5a, thrombin-cleaved osteopontin, and bradykinin [26]. A similar positive association between TAFI and CLT has been reported in patients with cardiovascular disease and those with venous thrombosis [26–28]. To the best of our knowledge, this study is the first to demonstrate that increased TAFI could contribute to hypofibrinolysis in AS. In the AS group TAFI remained at the same level until it transiently decreased 1 h after exercise to re-increase beyond its initial value 24 h after exercise, while in controls TAFI activity levels decreased permanently since peak exercise and were lower than in AS at all time points. In AS patients we did not observe any exercise-related changes in TAFI antigen. In control group TAFI antigen decreased, which is in accordance with another study [29]. Methods that utilize antibodies specific for TAFI antigen quantify overall TAFI consumption and/or TAFI generation, and cannot measure the level of TAFI activity. Quantifying the extent of TAFI activation is a more precise diagnostic marker in thrombotic diseases [26]. Considering a similar profile in post-exercise changes in TAFI activity and CLT among AS patients (Fig. 1B and C), it could be presumed that elevated TAFI activity is largely responsible for delayed and transitory hyperfibrinolytic response in this disease. We observed slightly lower PAI-1 after exercise among controls and no alteration in AS patients, which indicates that this inhibitor does not contribute to altered pattern of CLT changes during exercise test. The data on post-exercise changes in plasma PAI-1 are inconsistent [30], but most of them showed a neutral effect [29]. It has to be underscored that since patients with

documented CAD, carotid artery stenosis, and peripheral artery disease were excluded, concomitant advanced atherosclerosis, known to be associated with prolonged CLT [31], cannot explain attenuated fibrinolytic response to exercise in our AS group. Interestingly, our data indicate that intense exercise does not positively affect hemostasis in moderate-to-severe AS patients. If indeed exercise-induced prothrombotic alterations in circulating blood are clinically relevant, such prothrombotic phenotype might be an additional argument for earlier surgical treatment. This concept merits validation in a large prospective study.

Endothelial cell activation

A profile of changes in soluble TM in AS, which has been reported here for the first time, deserves a comment. We found that TM remained unchanged in AS during and after exercise, while it decreased in the current age-matched controls; in healthy individuals most studies showed no exercise-related changes of TM levels [32,33]. TM is involved both in the suppression of thrombin generation and impairment of fibrinolysis via activation of TAFI. Rapid turbulent blood flow in valve stenosis can damage endothelial cells lining the ascending aorta [31] which results in increased shedding of TM from the endothelium. Sustained high TM levels could be involved in unaltered TAFI levels post-exercise in AS.

Study limitations

Both study groups were small, however their size was sufficient to show the intergroup differences in fibrinolytic parameters. Our findings cannot be likely extrapolated to the whole population of AS patients since the study participants with mild or symptomatic AS were excluded. The patients studied had no bleeding tendency and multimers of vWF were not measured, therefore the impact of exercise test on this parameter remains to be established. Follow-up larger studies are needed to assess the risk of cardiovascular events in AS and their links with disturbed hemostasis. This issue was beyond the scope of the present study.

Conclusions

In conclusion, our results indicate that asymptomatic patients with moderate or severe AS respond to physical activity with a marked increase in endogenous thrombin potential and suppressed fibrinolytic capacity compared with controls. The present data combined with previous observations [11] suggest that exercise-induced prothrombotic alterations in AS patients may promote fibrin deposition on aortic valve leaflets, leading to the progression of this disease. It might be speculated that intense exercise could contribute to faster progression of valvular lesions and higher risk of cardiovascular events. The potential clinical relevance of altered hemostasis in AS warrants further investigation.

Conflict of interest

None declared.

Acknowledgments

The authors thank the technical and nursing staff at the Day Care Unit in the John Paul II Hospital, Krakow.

They also acknowledge that this research was supported by a grant of the Jagiellonian University Medical College (Grant numbers: N402 38338 and K/ZDS/002936 to AU).

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