



# Endothelial dysfunction in patients with eosinophilic granulomatosis with polyangiitis

Renata Pacholczak<sup>1,2</sup> · Stanisława Bazan-Socha<sup>3</sup> · Teresa Iwaniec<sup>3</sup> · Lech Zaręba<sup>4</sup> · Stan Kielczewski<sup>1</sup> · Jerzy Andrzej Walocha<sup>1</sup> · Jacek Musiał<sup>3</sup> · Jerzy Dropiński<sup>3</sup>

Received: 17 March 2018 / Revised: 9 July 2018 / Accepted: 2 August 2018 / Published online: 21 August 2018  
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## Abstract

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare form of vasculitis associated with asthma and eosinophilia. Endothelial dysfunction has been well documented in other types of vasculitis but not in EGPA. Thirty patients (10 men and 20 women) diagnosed with EGPA and remaining in a remission, and 58 controls (24 men and 34 women) matched for age, sex, and body mass index, were enrolled in the study. We assessed each participants for typical risk factors of cardiovascular diseases and measured serum levels of vascular cell adhesion molecule-1 (VCAM-1), interleukin 6 (IL-6), and thrombomodulin. We also measured flow-mediated dilatation (FMD) of the brachial artery and intima-media thickness (IMT) of the common carotid artery using ultrasonography. Patients with EGPA had 20% higher serum level of VCAM-1 ( $p < 0.001$ ) and 41.9% of thrombomodulin ( $p < 0.001$ ). They also had 38.8% lower relative increase of FMD (FMD%) ( $p < 0.001$ ), indicating endothelial dysfunction. These differences remained significant also after adjustment for potential confounders. Laboratory and ultrasonographic parameters of endothelial injury were correlated to the markers of inflammation and impaired kidney function. Determinants of lower FMD% in a simple regression model were pack-years of smoking ( $\beta = -0.3$  [95% confidence interval (CI)  $-0.5$  to  $-0.1$ ]), serum level of IL-6 ( $\beta = -0.36$  [95% CI  $-0.62$  to  $-0.1$ ]), and thrombomodulin ( $\beta = -0.34$  [95% CI  $-0.6$  to  $-0.08$ ]). EGPA patients are characterized by inflammatory endothelial injury that is likely related to the pathogenesis of the disease. Proper immunosuppressive treatment is the best method to prevent atherosclerosis and future cardiovascular events, the patients may also benefit from additional preventive interventions.

**Keywords** Atherosclerosis · Endothelium · Eosinophilic granulomatosis with polyangiitis · FMD

## Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA, formerly named Churg-Strauss syndrome) is a rare disease with

a prevalence that ranges from 10.7 to 13 cases per million [1]. Although EGPA is considered to be an anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), a significant ANCA serum concentration is found only in approximately 40% of patients, usually in the perinuclear immunofluorescence pattern (p-ANCA) [2]. Tissues affected show necrotizing vasculitis in EGPA patients who are ANCA-positive while in those without ANCA, the vessel walls are infiltrated mainly by eosinophils [3]. Due to the lack of specificity of antibodies, diagnosis of EGPA is generally made clinically. However, a biopsy of the affected organ may reveal some typical features, such as the extravascular granulomas, small- and medium-sized vessels vasculitis, and the eosinophilic infiltrates [1]. In contrast to the other types of AAV, patients with EGPA characteristically suffer from asthma with eosinophilia, the hallmark of this disease, occurring in over 95% of individuals [4, 5]. Other symptoms include nasal polyps, lung infiltrates, and mononeuritis multiplex [6]. As

✉ Jerzy Dropiński  
jerzy.dropinski@uj.edu.pl

<sup>1</sup> Department of Anatomy, Jagiellonian University Medical College, Cracow, Poland

<sup>2</sup> Centre of Oncology, Maria Skłodowska-Curie Memorial Institute Cracow Branch, Cracow, Poland

<sup>3</sup> Second Department of Internal Medicine, Jagiellonian University Medical College, Cracow, Poland

<sup>4</sup> Faculty of Mathematics and Natural Sciences, University of Rzeszow, Rzeszow, Poland

EGPA is a systemic disease, clinical manifestation can be highly variable and patients with EGPA may also present with gastrointestinal, neurological, and cardiovascular symptoms [7–9].

In ANCA-positive AAV, vascular damage is caused by the stimulation of neutrophils by antibodies, their subsequent adhesion and migration to endothelium, release of proteolytic enzymes, and proinflammatory cytokines, leading to endothelial cell damage [3]. In the 60% of EGPA patients who are ANCA-negative, the exact pathology is unknown, but it is suspected to be related to impaired eosinophil function. A few studies have found endothelial damage and progression of atherosclerosis in AAV patients [10–13] but the significance of their results is limited by small sample size. We have found no such investigation specifically concerning patients with EGPA in the literature.

In this study, we sought to evaluate the endothelium function in subjects with EGPA. We performed a comprehensive analysis using laboratory and ultrasonographic tests. The inflammatory state was estimated by interleukin-6 (IL-6) serum level, while injury of endothelium was assessed by serum concentration of vascular cell adhesion molecule-1 (VCAM-1) and thrombomodulin. Endothelial function was determined by ultrasound measurements of flow-mediated dilatation (FMD) of a brachial artery. This method enables estimation of the endothelium's response to shear, releasing nitric oxide and dilatation. Progression of atherosclerosis was assessed by intima-media thickness (IMT) measurements of the common carotid arteries.

## Methods

### Participants

Our study is based on the case-control observational format. We received approval from the Bioethics Committee of Jagiellonian University Medical College. All study participants were given a thorough explanation of the methodology and safety protocol before giving written consent for their inclusion in the study.

The case group was made up of 30 patients with EGPA—20 women and 10 men. They were recruited in the period from 2014 to 2017 at the Department of Allergy and Clinical Immunology at the University Hospital in Cracow, a diagnostics and treatment center for vasculitis in southern Poland. The patients were all previously diagnosed with EGPA based on the criteria of the American College of Rheumatology [14]. The activity of the disease was measured using the Birmingham Vasculitis Activity Score (BVAS) [15]. We included only patients remaining in a remission of the disease. Remission was defined as an absence of disease activity attributable to active disease qualified by the need for ongoing

stable maintenance immunosuppressive therapy [16]. Patients were excluded from the study if they also had angina pectoris, congestive heart failure, uncontrolled hypertension, liver failure, or cancer.

The control group was made up of 58 healthy individuals—34 women and 24 men matched to the EGPA group by gender, age, and body mass index [BMI]. Exclusion criteria were any manifestation of atherosclerosis, arterial hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, congestive heart failure, liver injury, chronic kidney disease, smoking (current or more than 1 year in the past), and a positive family history of cardiovascular diseases. The criteria for each comorbidity were as follows: arterial hypertension, a history of hypertension (blood pressure > 140/90 mmHg) or ongoing antihypertensive therapy; hypercholesterolemia, a serum total cholesterol > 5.2 mmol/l or ongoing antihypercholesterolemic therapy; hypertriglyceridemia, a serum triglycerides > 1.7 mmol/l; diabetes mellitus, use of insulin or oral hypoglycemic agents or a fasting serum glucose > 7.0 mmol/l; congestive heart failure, left ventricular ejection fraction below 40%; liver injury, a serum alanine aminotransferase elevated more than twice above upper limit of the reference range; and chronic kidney failure, an estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73 m<sup>2</sup>.

### Laboratory analysis

Fasting blood samples were drawn in the morning from the antecubital vein with minimal tourniquet use. Routine laboratory techniques were used to measure lipid profile, glucose, alanine transaminase, urine, creatinine for eGFR, and complete blood count (including eosinophilia and platelet count). C-reactive protein (CRP) was measured by the Johnson & Johnson VITROS 250. Blood samples were drawn into serum separation tubes, centrifuged at 2000×g for 10 min at room temperature within 2 h of collection. The supernatant was frozen in aliquots and stored at –70 °C until analyzed. IL-6, VCAM-1, and soluble thrombomodulin levels were measured using standardized ELISA assay (R&D Systems, Minneapolis, MN, USA). Antinuclear antibodies (ANA) and ANCA were analyzed in patients by indirect immunofluorescence tests (ThermoFisher, Waltham, USA). Sera with positive ANA or ANCA were further analyzed with antigen-specific ELISA for autoantibodies against proteinase 3 (anti-PR3 IgG), myeloperoxidase (anti-MPO IgG) (EUROIMMUN, Lübeck, Germany).

### Spirometry

Spirometry was performed using a Jaeger Master Screen spirometer on patients with EGPA according to the standards of the American Thoracic Society.

## Ultrasound examinations

Ultrasound studies were performed in a darkened, quiet room with subjects resting in a supine position for 10 min prior and fasting for 10 h. Examinations were conducted by two independent ultrasound experts using the Siemens Acuson Sequoia 512 with a 10-MHz linear array ultrasonic transducer (MountainView, CA, USA). Both experts made three consecutive measurements of each parameter (described below). The value used for each parameter was the mean of the six measurements. In addition, a transthoracic echocardiogram (TTE) was performed for every participant with measurements of left ventricular ejection fraction (EF) and of systolic pulmonary artery pressure using standard methods [17].

## Brachial artery ultrasonography

Flow-mediated dilatation (FMD) of the brachial artery was measured using Celermayer's method [18]. A baseline sagittal diameter (D1) of a distal part of the brachial artery was measured in the M-presentation by using a 10-MHz linear array ultrasonic transducer placed 2–3 cm proximal to the arterial bifurcation. A sphygmomanometer cuff was then placed on the forearm below the elbow and inflated to a pressure of 200 mmHg for 5 min and released. One minute after releasing the cuff, the brachial artery diameter was measured again (D2) at the same point. FMD was defined as the increase of brachial artery diameter after deflation of the cuff and was expressed as a percentage of the baseline diameter ( $\text{FMD}\% = [(D2 - D1) / D1] \times 100\%$ ).

## Intima-media thickness of the common carotid artery

The intima-media thickness (IMT) of the carotid artery was measured by ultrasound with a 10-MHz linear transducer. The thickness of the anterior and posterior walls of common carotid arteries was measured bilaterally in the longitudinal projection immediately proximal to their bifurcation. The mean value of the right and left thicknesses was used in further analysis.

## Statistical analysis

The results of the EGPA and control groups were compared using STATISTICA 12.5 software. The continuous variables were all non-normally distributed according to the Shapiro-Wilk test. They are reported here as median and interquartile range and compared using the Mann-Whitney *U* test. Categorical variables are reported as percentages and compared by  $\chi^2$  test. Potential confounders are age, sex, BMI, and comorbidities, such as arterial hypertension, diabetes

mellitus, and hypercholesterolemia. To adjust for these, the results of FMD%, IMT, VCAM-1, and thrombomodulin were log-transformed and a one-way covariance analysis (ANCOVA) was performed, first with an adjustment for age, sex, and BMI and subsequently to comorbidities. The univariate linear regression models with adjustment for BMI, age, and sex were used to analyze associations between two selected parameters. Unconditional multivariate logistic regression was performed to calculate odds ratios (ORs) with 95% confidence intervals (CIs). The cut-off values for VCAM-1, FMD%, and thrombomodulin were determined by receiver operating characteristic (ROC) curves. Determinants of FMD% were assessed using simple regression models. Results were considered statistically significant when the *p* value was less than 0.05.

## Results

### Characteristics of patients and controls

Demographic and laboratory characteristics of patients with EGPA and controls are presented in Table 1. Both groups were similar in age, sex, and BMI. Patients with EGPA had higher eosinophilia and inflammatory markers, such as white blood cells, CRP, and IL-6. They had also higher triglycerides level, while healthy individuals had increased low-density lipoprotein. The remaining basic laboratory parameters were comparable between both analyzed groups. In TTE, subjects with EGPA had lower ejection fraction; the values of the remaining parameters were similar in patients and controls (Table 1).

In Table 2, we have shown parameters determining characterization of the EGPA at the time of evaluation. The median duration of the disease was 4.5 years (range 3–8) and all the patients were in remission. Almost all of the subjects were diagnosed with asthma (96.7%, *n* = 29), with low to moderate bronchial obstruction in spirometry. Inflammation of the paranasal sinuses was found in 24 (80%) subjects and nasal polyps in 14 (46.7%). Heart involvement was documented in 66.7% (*n* = 20) of patients, 13 (43.3%) had lung infiltrates, and the same number was diagnosed with peripheral nerve damage.

Positive ANCA were detected in 53.3% of patients (*n* = 16), ANA in 33.3% (*n* = 10), and anti-MPO in 23.3% (*n* = 7), while anti-PR3 was present only in one subject.

Among patients with EGPA, 86.7% (*n* = 26) were currently being treated with corticosteroids (at a median dose equivalent to 8 mg/day of methylprednisolone, range 4–16). They also received immunosuppressive therapy in the past: azathioprine (30%, *n* = 9), cyclophosphamide (43.3%, *n* = 13), methotrexate (16.7%, *n* = 5), and mycophenolate mofetil or rituximab (both 3.3%, *n* = 1). Comorbidities in EGPA patients

**Table 1** A summary of demographic, laboratory, and echocardiographic parameters in patients with eosinophilic granulomatosis with polyangiitis and controls

	Patients, <i>n</i> <sup>a</sup> = 30	Controls, <i>n</i> = 58	<i>p</i> value
Age (years)	49 (44–58)	47.5 (40.5–58.5)	0.63
Male gender, number (%)	10 (33.3)	24 (41.4)	0.36
Female gender, number (%)	20 (66.6)	34 (58.8)	0.72
Body mass index (kg/m <sup>2</sup> )	24.9 (21.2–27.5)	26.6 (23.9–29.1)	0.1
Basic laboratory tests			
Hemoglobin (g/dl)	13.4 (12.3–14.7)	13.7 (12.7–15)	0.42
Red blood cells (106/μl)	4.5 (4.1–5.0)	4.5 (4.2–4.9)	0.67
White blood cells (103/μl)	8.6 (6.96–13.1)	5.9 (5.03–6.96)	0 < 001*
Blood eosinophilia (103/μl)	0.3 (0.1–0.9)	0.1 (0.09–0.2)	0.02*
Blood platelet count (103/μl)	242 (187–327)	225 (200–275)	0.36
Total cholesterol (mmol/l)	5.4 (4.3–6)	4.9 (4.2–5.3)	0.12
Low-density lipoprotein (mmol/l)	2.5 (2.3–3.1)	3.1 (2.5–3.6)	0.02*
Triglycerides (mmol/l)	1.4 (1.1–2.1)	1.1 (0.7–1.5)	0.01*
Glucose (mmol/l)	4.7 (4.1–5.1)	4.95 (4.7–5.2)	0.07
Creatinine (mmol/l)	71.2 (59–81)	76.1 (68.3–90)	0.06
Urea (mmol/l)	5.3 (3.93–6.3)	4.6 (3.9–5.3)	0.15
Estimated glomerular filtration rate (ml/min/1.73 m <sup>2</sup> )	81 (60–102.5)	60 (60–80)	0.03*
Alanine transaminase (IU/l)	26 (21–34)	22.5 (14–28)	0.05
C-reactive protein (mg/dl)	5 (1.7–11.1)	1.2 (1–2.1)	0 < 001*
Interleukin-6 (pg/ml)	4.5 (1.7–9.4)	1.7 (1.1–2.2)	0 < 001*
Echocardiographic parameters			
Left ventricular diastolic diameter (cm)	4.7 (4.4–4.9)	4.7 (4.5–4.9)	0.43
Left ventricular systolic diameter (cm)	3 (2.9–3.2)	3 (2.9–3.1)	0.55
Right ventricular diameter (cm)	2.1 (2–2.3)	2.1 (1.9–2.3)	0.42
Left atrial diameter (cm)	3.7 (3.4–3.8)	3.7 (3.5–3.9)	0.43
Left ventricle posterior wall thickness (cm)	1 (0.9–1)	0.9 (0.8–1)	0.19
Interventricular septum thickness (cm)	1 (0.9–1.1)	0.9 (0.8–1)	0.15
Ejection fraction (%)	62.5 (50–68)	68 (68–70)	0 < 001*
Pulmonary artery pressure (mmHg)	33 (30–36)	32 (26–32)	0.06
Laboratory parameters of endothelial injury			
Vascular cell adhesion molecule-1 (ng/ml)	966.1 (845.8–1129.5)	804.6 (694.4–936.7)	0 < 001*
Thrombomodulin (ng/ml)	6.1 (5.2–6.5)	4.3 (3.9–4.7)	0 < 001*
Ultrasound parameters of endothelial injury and atherosclerosis			
Relative increase of flow-mediated dilatation of a brachial artery (%)	6.3 (5.3–8.8)	10.3 (8.9–12.5)	0 < 001*
Median value of intima-media thickness of a common carotid artery (cm)	0.07 (0.06–0.08)	0.07 (0.06–0.08)	0.24

Categorical variables are presented as numbers (percentages) and continuous variables as median and interquartile range. The results which are statistically significant are marked \*

<sup>a</sup> *n* number

included arterial hypertension (23.3%, *n* = 7), diabetes mellitus (10%, *n* = 3), and hypercholesterolemia (26.7%, *n* = 8) (Table 2). Angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists were taken by 36.7% of patients (*n* = 11), beta-blockers and diuretics by 26.7% (*n* = 8), and statins by 20% (*n* = 6), while calcium channel blockers were used in 13.3% (*n* = 4) of subjects. Ten subjects (33%) were ex-smokers.

### Laboratory markers of endothelial injury

Patients with EGPA had a 20% higher serum level of VCAM-1 and a 41.9% of thrombomodulin compared to healthy individuals (both *p* < 0.001). In ANCOVA analysis, we demonstrated that both these markers remained increased in EGPA subjects also after adjustment for potential confounders (age, sex, BMI, hypercholesterolemia, hypertension, and diabetes

**Table 2** Clinical characteristics of the patients ( $n = 30$ ) with eosinophilic granulomatosis with polyangiitis

	Patients
Duration of the disease (years)	4.5 (3–8)
Antinuclear antibodies presence, $n^a(\%)$	10 (33.3)
Anti-neutrophil cytoplasmic antibodies presence, $n (\%)$	16 (53.3)
Anti-proteinase 3 IgG presence, $n (\%)$	1 (3.33)
Anti-myeloperoxidase IgG presence (%)	7 (23.3)
Organ involvement	
Asthma, $n (\%)$	29 (96.7)
Cutaneous vasculitis, $n (\%)$	7 (23.3)
Granulomatous lesions in ears/hearing disturbances, $n (\%)$	1 (3.3)
Nasal polyps, $n (\%)$	14 (46.7)
Paranasal sinuses inflammation, $n (\%)$	24 (80)
Bone destruction of paranasal sinuses, $n (\%)$	7 (23.3)
Chronic kidney disease, $n (\%)$	2 (6.7)
Lungs infiltrates, $n (\%)$	13 (43.3)
Peripheral nerves damage, $n (\%)$	13 (43.3)
Gastrointestinal symptoms, $n (\%)$	6 (20.0)
Heart involvement, $n (\%)$	20 (66.7)
Comorbidities	
Hypertension, $n (\%)$	7 (23.3)
Diabetes mellitus, $n (\%)$	3 (10.0)
Hypercholesterolemia, $n (\%)$	8 (26.7)
Spirometry	
FEV1% <sup>b</sup>	77.8 (64.6–94.5)
VC% <sup>c</sup>	93.9 (86.9–108.3)
FEV1/VC%	69.5 (57.7–79.7)
Smoking	
Currently, $n (\%)$	2 (6.7)
In the past, $n (\%)$	10 (33.3)
Smoking (packs/years)	0 (0–2.5)
Treatment characteristic	
Current steroids, $n (\%)$	26 (86.7)
Current corticosteroid dose, mg per day, recalculated to methylprednisolone	8 (4–16)
Systemic steroids therapy (years)	4 (2–6)
Immunosuppressive treatment (in the past)	
Azathioprine, $n (\%)$	9 (30.0)
Cyclophosphamide, $n (\%)$	13 (43.3)
Methotrexate, $n (\%)$	5 (16.7)
Mycophenolate mofetil, $n (\%)$	1 (3.3)
Rituximab, $n (\%)$	1 (3.3)
Internal medicine medications	
Angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists, $n (\%)$	11 (36.7)
Statins, $n (\%)$	6 (20.0)
Beta-blockers, $n (\%)$	8 (26.7)
Diuretics, $n (\%)$	8 (26.7)
Calcium channel blockers, $n (\%)$	4 (13.3)

Categorical variables are presented as numbers (percentages) and continuous variables as median and interquartile range

<sup>a</sup>  $n$  number

<sup>b</sup> FEV1% forced expiratory volume in 1 s, percentage of reference range

<sup>c</sup> VC% vital capacity, percentage of reference range

mellitus) (both,  $p < 0.001$ ). EGPA subjects were characterized by a 2.63 [95% CI 1.13–5.17] increased risk of elevated VCAM-1 and a 4.68 [95% CI 2.3–9.5] risk of higher thrombomodulin, as compared to the controls (cut-off points 837.6 ng/ml and 5.2 ng/ml, respectively). Interestingly, both endothelial injury parameters were negatively associated with the duration of the disease ( $\beta = -0.66$  [95% CI  $-0.9$  to  $-0.41$ ],  $\beta = -0.43$  [95% CI  $-0.79$  to  $-0.07$ ] VCAM-1 and thrombomodulin, respectively).

The level of thrombomodulin, but not VCAM-1, was positively related to the inflammatory markers, such as IL-6 ( $\beta = 0.52$  [95% CI 0.17–0.87]), CRP ( $\beta = 0.58$  [95% CI 0.17–0.99]), and white blood cell count ( $\beta = 0.39$  [95% CI 0.06–0.72]). We found positive associations between VCAM-1 and urea or creatinine blood levels ( $\beta = 0.49$  [95% CI 0.21–0.77] and  $\beta = 0.36$  [95% CI 0.07–0.65], respectively). We also documented similar relationships for thrombomodulin ( $\beta = 0.39$  [95% CI 0.04–0.74],  $\beta = 0.69$  [95% CI 0.41–0.97], urea and creatinine blood



levels, respectively). Additionally, VCAM-1 in patients with EGPA was related to the interventricular septum and posterior wall thickness ( $\beta = 0.52$  [95% CI 0.29–0.75],  $\beta = 0.63$  [95% CI 0.42–0.84], respectively). Comorbidities, medications, and type of immunosuppressive treatment in the past had no impact on laboratory markers of endothelial damage in the EGPA group after comparison in subgroups.

### Ultrasound parameters of endothelial injury

The EGPA group was characterized by a 38.8% decrease in FMD%, compared to the control group ( $p < 0.001$ ), also after adjustment for potential confounders (age, sex, BMI, hypercholesterolemia, hypertension, and diabetes mellitus), and higher risk of diminished FMD% defined as values below the cut-off point of 8.51 (OR 3.8 [95% CI 2.2–6.56]). FMD% was negatively associated with CRP level ( $\beta = -0.5$  [95% CI -0.75 to -0.25]) without associations with remaining inflammatory markers. However, a simple regression models showed that serum level of IL-6 ( $\beta = -0.36$  [95% CI -0.62 to -0.1]) and thrombomodulin ( $\beta = -0.34$  [95% CI -0.6 to -0.08]) may be related to lower FMD% values. The same relationship was found for pack-years of smoking ( $\beta = -0.3$  [95% CI -0.5 to -0.1]).

Among patients with EGPA, a lower FMD% was observed in those with polyneuropathy (5.7 [5.1–6.2] vs. 8.3 [6.3–9.5],  $p = 0.01$ ) without differences to the other subgroups regarding comorbidities or clinical symptoms.

The IMT measurement was similar in the EGPA and control groups and it did not correlate with laboratory parameters of endothelial injury.

### Discussion

This study is the first to find that EGPA is characterized by endothelial injury. We demonstrated that even when EGPA is in remission, increased levels of thrombomodulin and VCAM-1 are present in the blood, suggesting ongoing endothelial damage. Moreover, laboratory markers of endothelial injury were accompanied by functional abnormalities in vasodilatation of the brachial artery without acceleration of atherosclerosis reflected by IMT values. We also found a positive correlation between VCAM-1 and interventricular septum and posterior wall thickness, which indicate general stiffness not only of vessels but also of the heart structures. The evaluated parameters of endothelial damage did not differ regardless of comorbidities, medications, or immunosuppressive treatment in the past, as it has been shown in statistical analyses performed in this study.

Our findings are partly in the line with other studies regarding endothelial injury in vasculitis. Schimtt et al. [19] demonstrated that serum levels of thrombomodulin were

significantly elevated only during the active stage of EGPA and correlated with disease activity. However, anti-endothelial cell antibodies which may be one of the driving mechanisms for vascular injury in AAV were detected in EGPA patients regardless of the stage of the disease in their study. Sangle et al. [12] confirmed accelerated atherosclerosis in AAV patients (seven of them were EGPA subjects) by measurements of ankle-brachial pressure index. In the other study, performed by Chironi et al. [11], subclinical atherosclerosis was detected by ultrasonic measurements of plaque in three peripheral vessels (carotid and femoral arteries and abdominal aorta; in this study 11 EGPA patients were enrolled).

Several explanations for the mechanism of endothelial injury in EGPA have been proposed. Activated eosinophils secrete diverse pharmacologically active granule components with cytotoxic action capable of inducing damage of endothelial cells [20]. Eosinophil activation can be assessed by the detection of eosinophil cationic protein (ECP), major basic protein, and eosinophil peroxidase. All may accumulate on endothelial surfaces and have a potent damaging effect, directly and indirectly via the inhibition of the cell's ability to support thrombin-dependent activation of protein C [21]. It has also been shown that ECP can bind to Hageman factor (coagulation factor XII) in vitro and cause activation of the intrinsic coagulation pathway [20]. Notably, in a retrospective study assessing the role of eosinophils in thrombosis, 56% of patients with EGPA presented in medical history with venous thrombosis, 38% with arterial thrombosis, and 4% with mixed thrombosis [22]. In a study by Kain et al. [23], over 90% of enrolled patients with autoimmune diseases had autoantibodies to lysosomal membrane protein-2 that cause injury to endothelial cells in vitro. This may explain ANCA negativity in some AAV patients. In ANCA-positive subjects, the contact of neutrophils and ANCAs with endothelium is considered to be the key event in endothelial injury. In our study, the parameters of endothelial injury did not differ in subgroups regarding presence of ANCA, ANA, and anti-MPO antibodies (data not shown).

An alternative possibility for explanation endothelial injury in EGPA is the influence of traditional cardiovascular risk factors, including smoking and comorbidities, such as arterial hypertension, diabetes mellitus, and hypercholesterolemia. Although in our study, the pack-year smoking history was an important factor affecting diminished FMD%, it had no impact on laboratory markers of endothelial injury. Moreover, in our study, no comorbidity had any effect on endothelial injury parameters. Chronic kidney failure is another factor that is thought to lead to endothelial dysfunction in EGPA as it does in other autoimmune diseases [24]. This hypothesis is partly supported in our study, reflected by the positive correlation between VCAM-1 and thrombomodulin with the serum levels of creatinine and urea. However, only 7% of EGPA patients enrolled in our study had confirmed chronic kidney disease. Moreover, the presence of this

complication can be attributed to a manifestation of EGPA in these patients and may not even be a separate comorbidity.

Another mechanism is the atherogenic effect of systemic inflammation. This is suggested in our study by the inverse associations of white blood cells, CRP, and IL-6 with laboratory parameters of endothelial damage in the overall population of enrolled patients and controls (data not shown). Because the duration of the disease was negatively related to the CRP concentration (data not shown), we concluded that a longer disease course that is well managed with corticosteroids and immunosuppressants is a less active disease in terms of acute inflammation. This may explain our results of negative associations between VCAM-1, thrombomodulin, and the duration of the disease. Other studies found that only the active phase of vasculitis accelerates atherosclerosis and immunosuppressive treatment is the best method to control not only the disease but also to prevent endothelial injury [25, 26]. Gonzalez-Juanatey et al. [27] demonstrated significant improvement of FMD% 4 weeks after the onset of steroid therapy. Interestingly, other authors have shown that immunosuppressive treatment, especially with cyclophosphamide and azathioprine, directly injures endothelial cells [28, 29]. The number of patients in our study was too small to allow reliable subgroup analysis in terms of immunosuppressive treatment. Additionally, the majority of our patients with EGPA were also treated with steroids. Prolonged steroid therapy is associated with hypertension, diabetes mellitus, and change in the lipid profile, all of which influence on the risk of cardiovascular events. This may be partially offset by the finding that steroids also improve endothelial function [27].

Based on the aforementioned results, we conclude that immunosuppressive treatment is the best method to control acute inflammation and prevent endothelial injury. However, even in the remission phase of EGPA, patients undergo endothelial injury and would therefore benefit from additional preventive interventions such as aspirin or statins therapy.

Our study is limited by the small number of participants due to the relative rarity of the disease, not allowing for the recruitment of a sufficient number of patients. Our control group was not well matched in terms of comorbidities but we attempted to eliminate these confounding variables by an adjustment for comorbidities (hypercholesterolemia, hypertension, diabetes mellitus) during statistical analysis. FMD% and IMT are subjective measurements. To reduce the effect of inter-operator variability, we used the same two researchers to perform all the measurements and we considered mean of three subsequent measurements in a further analysis.

## Conclusion

Patients with EGPA present with endothelial injury which is mainly caused by the pathogenesis of the disease: the

damaging effect of eosinophils, anti-neutrophil antibodies, and inflammation. Although the process is mainly active in acute inflammation, patients in remission of the disease also present with ongoing endothelial injury. Proper immunosuppressive treatment is the best method to prevent atherosclerosis and future cardiovascular events; however, the patients may benefit from additional preventive interventions.

**Funding** This project was funded by the research grants of Jagiellonian University Medical College No: K/ZDS/007900 (to S.B.-S.) and No: K/DSC/004605 (to R.P., donation for young scientists).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Compliance with ethical standards

**Disclosures** None.

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