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NETosis is associated with the severity of aortic stenosis: Links with inflammation

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ABSTRACT

Background: An involvement of neutrophil extracellular traps (NETs) in the aortic stenosis (AS) pathogenesis is unknown.
Methods: We enrolled 50 patients, median age 66.5 years with isolated severe AS, after aortic valve replacement and 20 healthy sex/age-matched controls. Autopsy-derived aortic valves from 5 healthy donors served as controls. Valvular expression of citrullinated histone H3 (citH3), myeloperoxidase (MPO), and neutrophil elastase (NE) and macrophages (CD68) were evaluated by immunostaining. Plasma citH3 and interleukin 6 (IL-6) were also determined.
Results: All stenotic and healthy valves expressed citH3 in the leaflets' endothelial and sub-endothelial layers at the aortic side. Amount of valvular citH3-positive cells was higher in AS patients compared with controls (53.5 [33–62]% vs. 5.7 [4.1–9.0]%, $p < 0.0001$) and correlated with aortic valve area (AVA; $r = -0.69$, $p < 0.0001$) and mean transvalvular gradient ($r = 0.6$, $p < 0.0001$). Double-staining revealed that in AS valves 27.2 ± 9.8% of cells were citH3/MPO- and 25.3 ± 8.9% citH3/NE-positive. Within stenotic valves, 6.4 ± 2.5% of cells showed citH3/CD68 double-positivity and were identified as macrophages. AS patients compared to controls had 83% higher plasma citH3 ($p < 0.0001$). In AS, plasma citH3 correlated with IL-6 ($r = 0.39$, $p = 0.0054$) levels and AVA ($r = -0.45$, $p = 0.0009$).
Conclusions: The presence of NETs in stenotic valves and association with AS severity suggest novel mechanisms involved in the disease progression.

1. Introduction

Aortic stenosis (AS) is the most common cause of acquired valvular heart disease in the adult population, with no available pharmacological treatment to inhibit the disease progression [1,2]. AS is closely linked to atherosclerosis, at least in terms of risk factors and underlying pathomechanisms, namely chronic inflammation. Infiltration of the aortic valve leaflets by monocytes is followed by their differentiation into macrophages and the resulting calcification [3–7]. Growing evidence indicates an involvement of T cells, B cells, mast cells, and platelets in AS [8–11]. The neutrophil involvement in AS progression has not been shown, however recent studies demonstrated that enhanced hemodynamic forces, similar to those observed in AS patients, can activate neutrophils and induce release of chromatin-based structures called neutrophil extracellular traps (NETs) [12]. Neutrophils and NETs are emerging as important mediators of pathogenic inflammation and trigger blood coagulation, recruiting platelets and creating scaffolding upon which thrombus can be assembled [13]. Both inflammation and coagulation have been documented to be involved in AS development and progression [14], while the presence of NETs has been documented in human atherosclerotic vessels [15].

An involvement of NETs in atherosclerosis development is related to endothelial injury or dysfunction due to i.e. hypertension or lipids oxidation [16]. After tissue injury neutrophils migrate to the site of tissue damage and release NETs consisting of nuclear histones and neutrophilic proteins such as myeloperoxidase (MPO) and elastase (NE) [17]. Of note, Pérez-Sánchez et al. have demonstrated an association between increased level of cell-free nucleosomes and the carotid intima-media thickness as a marker of atherosclerosis progression [16]. Moreover, NETs stimulate macrophages to produce pro-inflammatory cytokines, including interleukin-1β (IL-1β), responsible for the recruitment of immune cells to aortic valves [18].

To our knowledge, there have been no reports investigating whether neutrophils/NETs are involved in the development and/or AS

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progression. Thus, we sought to evaluate NETosis in patients with AS. We hypothesize that NETs activated by either affected valvular endothelium or activated macrophages might contribute to AS progression.

2. Materials and methods

2.1. Patients

We enrolled 50 patients with isolated AS between February 2018 and August 2018 in the Department of Cardiovascular Surgery and Transplantology at the John Paul II Hospital, Krakow. All AS patients enrolled to our study were diagnosed in peripheral hospitals and were referred to our clinic when they became severe symptomatic AS patients. AS was defined as mean transvalvular gradient ≥40 mm Hg and/or aortic valve area (AVA) <1 cm^2 based on transthoracic echocardiography, made by an experienced cardiologist on a GE Vivid 7 (GE Healthcare, Chalfont St Giles, UK) ultrasound machine. Left ventricular ejection fraction (LVEF) was routinely measured during echocardiography. The exclusion criteria were: atherosclerosis, rheumatic AS, acute infection, diagnosed malignancy, endocarditis, diabetes mellitus, chronic kidney disease, previous heart surgeries in the last 3 months before inclusion, mitral valve surgery, heart attack, stroke, and pregnancy. The diagnosis of atherosclerosis was based on angiographically documented coronary artery stenosis >20% diameter. Hypercholesterolemia was diagnosed based on medical records, cholesterol-lowering therapy or total cholesterol of 5.0 mmol/L or more. Arterial hypertension was diagnosed based on a history of hypertension (blood pressure > 140/90 mm Hg) or preadmission antihypertensive treatment. Smoking was defined as the use of one or more cigarettes per day [19]. The study was designed as the group-matched. The control group comprised 20 healthy, age- and sex-matched volunteers to assess plasma concentrations of citrullinated histone H3 (citH3) and interleukin-6 (IL-6) levels. Aortic valves obtained at autopsy from age-matched apparently healthy subjects (n = 5), without morphological valvular or other cardiac disorders served as negative control. The Jagiellonian University Medical College Ethical Committee approved the study, and participants provided informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

2.2. Laboratory analysis

After an overnight fast venous blood drawn between 7:00 and 9:00 AM before valve replacement from the antecubital vein into citrated tubes (9:1 of 0.106 M sodium citrate) was centrifuged at 2500*g* at 20 °C for 20 min and stored in aliquots at −80 °C until analysis. Blood drawn into serum tubes was centrifuged at 1600*g* at 4 °C for 10 min and stored at −80 °C. To determine lipid profile, glucose and creatinine, routine laboratory assays were used. High-sensitivity C-reactive protein (CRP) was determined using immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). Fibrinogen was measured by the von Clauss method.

2.3. Enzyme-linked immunosorbent assay (ELISA)

CitH3 and IL-6 serum levels were determined using commercial ELISA kits (BlueGene Biotech, Shanghai, China and R&D System, Minneapolis, MN, USA, respectively) following the manufacturers' instructions.

2.4. Aortic valves preparation

Valve samples embedded in Tissue Tek-OCT compound (Sakura, Torrance, CA, USA) were cryosectioned (5 μm) onto SuperFrost slides (Menzel-Glaser, Braunschweig, Germany) by a Leica CM 1520 cryostat. Transverse sections were taken from the mid and commissural areas of the leaflet and stored at −20 °C until immunostaining.

2.5. Immunofluorescence analysis

Valvular sections prepared as previously described [11] were stained using primary adequate antibodies overnight at 4 °C. Single-label fluorescence was performed using monoclonal antibodies against citH3 (1:250; Abcam, Cambridge, UK), MPO (1:1000; GeneTex, Irvine, CA, USA), NE (1:50; GeneTex, Irvine, CA, USA), and macrophages (CD68) (1:400; Abcam, Cambridge, UK). Primary antibodies were followed by the corresponding secondary antibodies conjugated with fluorochrome AlexaFluor 488 or AlexaFluor 594 (Abcam, Cambridge, UK) (1:1500) at room temperature for 1 h. Double-label immunofluorescence analyses were performed using the same antibodies. A negative control (without primary antibody incubation) was included routinely (Fig. 1A). Data were expressed as a percentage of positively-stained cells after adjustment for non-specific staining. The analyses were performed using Olympus BX 43 microscope equipped with software Cell Sense Standard (version 11.0.06). The number of immunopositive cells was quantified per 100 consecutive cells per each section and 3 sections per each valve were assessed giving 300 cells analyzed per each valve [11].

2.6. Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR). Normality of the data was assessed using the *Shapiro-Wilk test*. Categorical variables were presented as numbers and percentages and were compared by Pearson's χ^2 or Fisher's exact-test. Differences between 2 groups were compared using the Student's t-test for normally distributed continuous variables and for non-normally distributed continuous variables the Mann-Whitney *U* test was used. Associations between nonparametric or parametric variables were assessed by the Pearson's test. *p*-Value of <0.05 was considered statistically significant. All statistical analyses were performed using STATISTICA software Version 12.5 (StatSoft STATISTICA™, Poland).

3. Results

3.1. Characteristics of the studied groups

Baseline characteristics of AS patients and controls are shown in Table 1. AS patients and healthy volunteers did not differ with regard to demographic factors or cigarette smoking, except for lower body-mass index (BMI) observed in controls compared with AS patients ($p = 0.026$) (Table 1). AS patients more often had arterial hypertension and hypercholesterolemia, but did not differ in other clinical and demographic factors (Table 1). No intergroup differences were observed in laboratory parameters (Table 1).

3.2. Valvular NETs specific biomarkers

Immunofluorescence analysis revealed the presence of citH3-positive cells within aortic valves in both groups. All stenotic and healthy donors' valves showed citH3-positive staining in the endothelial and sub-endothelial layers at the aortic side of the leaflets (Fig. 1B, C). The percentage of citH3-positive cells was higher in AS valves compared to the control valves (53.5 [33–62]% vs. 5.7 [4.1–9.0]%, $p < 0.0001$, Fig. 2A). The valvular expression of both MPO and NE was observed within stenotic (Fig. 1D, E) but not control valves. Double staining revealed that within stenotic valves 27.2 ± 9.8% of cells were positive for both citH3 and MPO (Fig. 1F), while 25.3 ± 8.9% of cells were citH3 and NE positive. Of note, MPO/NE-positivity was observed in 93% of citH3-positive cells. Within stenotic valves, 6.4 ± 2.5% of cells showed double-positivity for both citH3 and CD68 and were identified as macrophages (Fig. 1G, H).

3.3. Plasma markers

AS patients were characterized by 83% higher plasma citH3 concentrations (12.24 [8.7–18.0] vs. 6.7 [4.4–8.6] ng/ml, $p < 0.0001$; Fig. 2B) compared to healthy volunteers, also after adjustment for body-mass index ($p = 0.0014$) and hypercholesterolemia ($p = 0.03$). Similarly, IL-6 level was 70% higher in AS compared with controls (4.6 [3.2–6.0] vs. 2.7 [2.3–2.9] pg/ml, $p < 0.001$). There were no age-, gender-, smoking- or drug-related differences in the citH3 levels in any of examined groups. Of note, plasma citH3 levels correlated with valvular citH3 amounts ($r = 0.42$, $p = 0.0027$).

3.4. citH3 associations

In AS patients, but not in controls, plasma IL-6 concentrations were positively correlated with citH3 (Fig. 3A) plasma levels. In AS patients the amount of valvular citH3 correlated with the disease severity measured as AVA (Fig. 3B) and mean transvalvular gradient (Fig. 3C). Moreover, in AS patients plasma citH3 levels were also associated with AVA (Fig. 3D).

4. Discussion

Our study is the first to demonstrate that in patients with AS without documented significant atherosclerotic vascular disease, NETs are present not only within the aortic valve leaflets giving about 25% of total endothelium/subendothelium cells number, but their biomarker citH3 is elevated in plasma compared to controls. Moreover, associations of valvular and plasma citH3 amounts with the disease severity, reflected

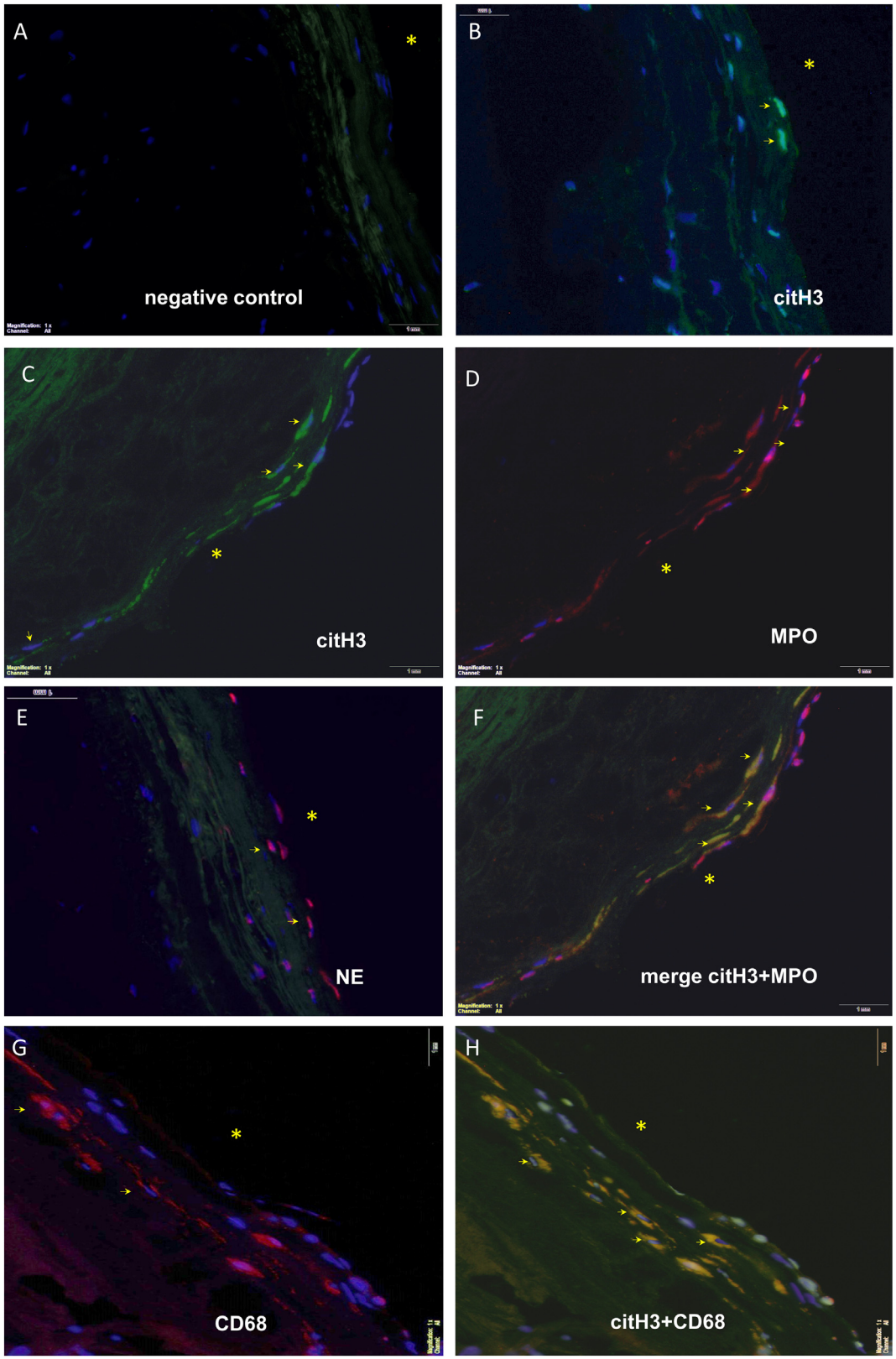

Fig. 1. Specific biomarkers of neutrophil extracellular traps (NETs) within stenotic aortic valve. (A) Negative control stained without citH3 primary antibody, (B) citrullinated histones (citH3, green) within a healthy valve, (C) citrullinated histones (citH3, green), (D) myeloperoxidase (MPO, red), and (E) neutrophil elastase (NE, red), (F) merged citH3 (green) and MPO (red) within aortic stenotic valves. (G) CD68 staining (red) and (H) double-staining of macrophages (CD68, red) and citH3 (green) performed to identify macrophages extracellular traps. Cell nuclei are stained in blue (DAPI). Colocalized areas of each factor are stained yellow and orange (combination of red and green). * aortic site of the leaflet. Original magnification 40×.

Table 1
Baseline characteristics of the study groups.

Variable	Patients with AS (n = 50)	Healthy volunteers (n = 20)	*p*-Value
Age, years	66.5 [58–69]	66 [63–68]	0.96
Female, n (%)	26 (52)	11 (55)	0.82
BMI, kg m^{-2}	28.4 ± 3.8	26.2 ± 3.5	0.026
Risk factors, n (%)			
Arterial hypertension	39 (78)	0	0.000
Hypercholesterolemia	35 (70)	9 (45)	0.047
Current smoking	6 (12)	3 (15)	0.5
Medications, n (%)			
Beta-blockers	30 (60)	0	0.0000
Acetylsalicylic acid	25 (50)	0	0.0000
Angiotensin-converting enzyme inhibitors	22 (44)	0	0.0001
Statins	34 (68)	0	0.0000
Echocardiographic parameters			
Mean gradient, mmHg	52.4 ± 12.2	NA	–
Maximum gradient, mmHg	83.4 ± 17.6	NA	–
LVEF, %	61 [60–66]	NA	–
AVA, cm^2	0.80 ± 0.18	NA	–
V max, m/s	4.9 ± 0.51	NA	–
Laboratory investigations			
Fibrinogen, g/L	3.3 [2.7–3.9]	3.3 [3.1–3.8]	0.61
Creatinine, μmol/L	75.6 ± 16.2	71.3 ± 8.4	0.41
hsCRP, mg/L	1.9 [1.0–2.2]	2.9 [1.8–3.4]	0.22
Glucose, mmol/L	5.5 [5.0–5.8]	5.4 [4.9–5.6]	0.61
TC, mmol/L	4.2 ± 1.1	4.8 ± 1.0	0.46
LDL-C, mmol/L	2.5 ± 1.0	3.4 ± 0.8	0.27
HDL-C, mmol/L	1.3 [1.1–1.7]	1.6 [1.3–1.9]	0.92
Triglycerides, mmol/L	1.3 [1.0–1.8]	1.6 [1.2–1.9]	0.29

Data presented as numbers (percentages), mean ± SD or medians (interquartile range). AS – aortic stenosis, BMI - body mass index, LVEF - left ventricular ejection fraction, AVA - aortic valve area, V max - maximum velocity, hsCRP - high-sensitivity C-reactive protein, TC - total cholesterol, LDL-C - low density lipoprotein cholesterol, HDL-C - high density lipoprotein cholesterol, NA - not applicable.

by echocardiographic parameters, might indicate novel link between valvular NETosis and AS progression.

AS is considered as an active inflammatory process that occurs in response to endothelial damage through high shear stress, which in our opinion could promote neutrophil influx and subsequent NETosis. At the same time, neutrophils and NETs are emerging as important mediators of pathogenic inflammation [13]. It has been shown that NETosis is promoted by IL-6 and IL-8, both expressed within stenotic valves [20–22]. Thus it is likely that proinflammatory cytokines released by activated valvular macrophages promote NETosis, which in turn could enhance valvular inflammation in a vicious cycle, contributing to valvular thickening, damage and calcification. It is possible that NETosis inhibition might slow down the AS progression.

It has been shown that platelets contribute to the progression of AS [10]. On the other hand, platelets have been described as active players in NETosis, through their interactions with charged extracellular histones [23]. Platelet activation and accumulation on NETs lead to increased permeability of endothelial monolayer, which might promote an influx of inflammatory cells within aortic valve leaflets [23]. Moreover, platelets recruited by NETs activate the coagulation cascade and serve as a scaffold upon which fibrin can be deposited enhancing inflammatory processes [13].

Taking into account similarities between atherosclerosis and AS, it seems important that a relationship between circulating cell-free DNA, NETosis, and atherosclerosis has been demonstrated. The severity of coronary artery disease was predicted by levels of cell-free DNA as well as a number of NET markers (nucleosomes, citH3, and MPO-DNA complexes) [24]. Data on the role of NETs in different diseases including rheumatoid arthritis, systemic lupus erythematosus, diabetes or ulcerative colitis demonstrate that in humans NETosis might be a therapeutic target by blocking this process or locally neutralizing NET signaling [25]. Moreover, in mice NETs were detected in close association with atherosclerotic plaques, while NETosis inhibitor - Cl-amidine decreased atherosclerotic lesion area in this model [26]. Further studies are needed to clarify the crosstalk between NETosis and fibro-calcification of aortic valve leaflets in AS.

We observed that only about 82% among citH3-positive cells were classified as neutrophils undergoing NETosis. Interestingly, about 18% of citH3-positive cells showed double-positivity for both citH3 and CD68 antigens identifying them as macrophages. It was reported that macrophages are able to release extracellular traps (METs) [27]. METs have been shown to capture and mediate clearance of bacteria, fungi, and parasites [28,29] and their formation was associated with the activation of elastase and NADPH oxidase [30]. However, this phenomenon has been poorly described so far. Our observation is the first to show that valvular macrophages in AS patients may release histones, but mechanism and importance of METosis in AS should be further explored.

Neutrophils and NETs have been linked to thrombosis [31]. The ability of neutrophils to expose functional tissue factor (TF) on NETs [32] is considered as a link between inflammation and coagulation. On the other hand, stenotic aortic valves exhibit the expression of TF and prothrombin [33–35]. In addition, enhanced expression of both coagulation and inflammatory proteins, i.e. TF, prothrombin, CRP, and IL-6, correlated with the severity of AS and the degree of valvular calcification [20,34,35]. Thus, valvular TF-bearing NETs might contribute to local microthrombosis and accelerate valvular damage resulting in the disease progression.

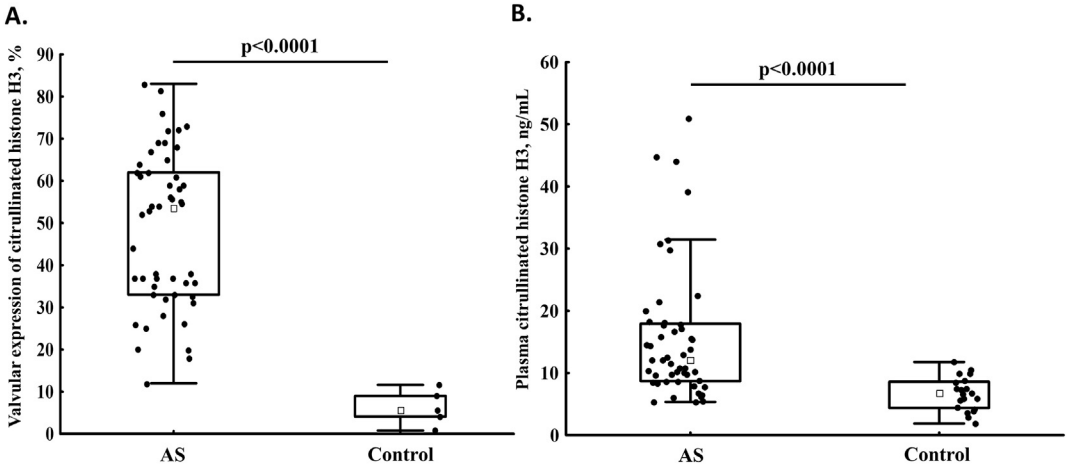

Fig. 2. Levels of citrullinated histone (citH3) in aortic stenosis (AS) patients and healthy subjects. (A) Semiquantitative analysis of immunostained citH3 expressed as median and interquartile range and (B) the level of plasma citH3 expressed as median and interquartile range. A *p*-value <0.05 was considered statistically different.

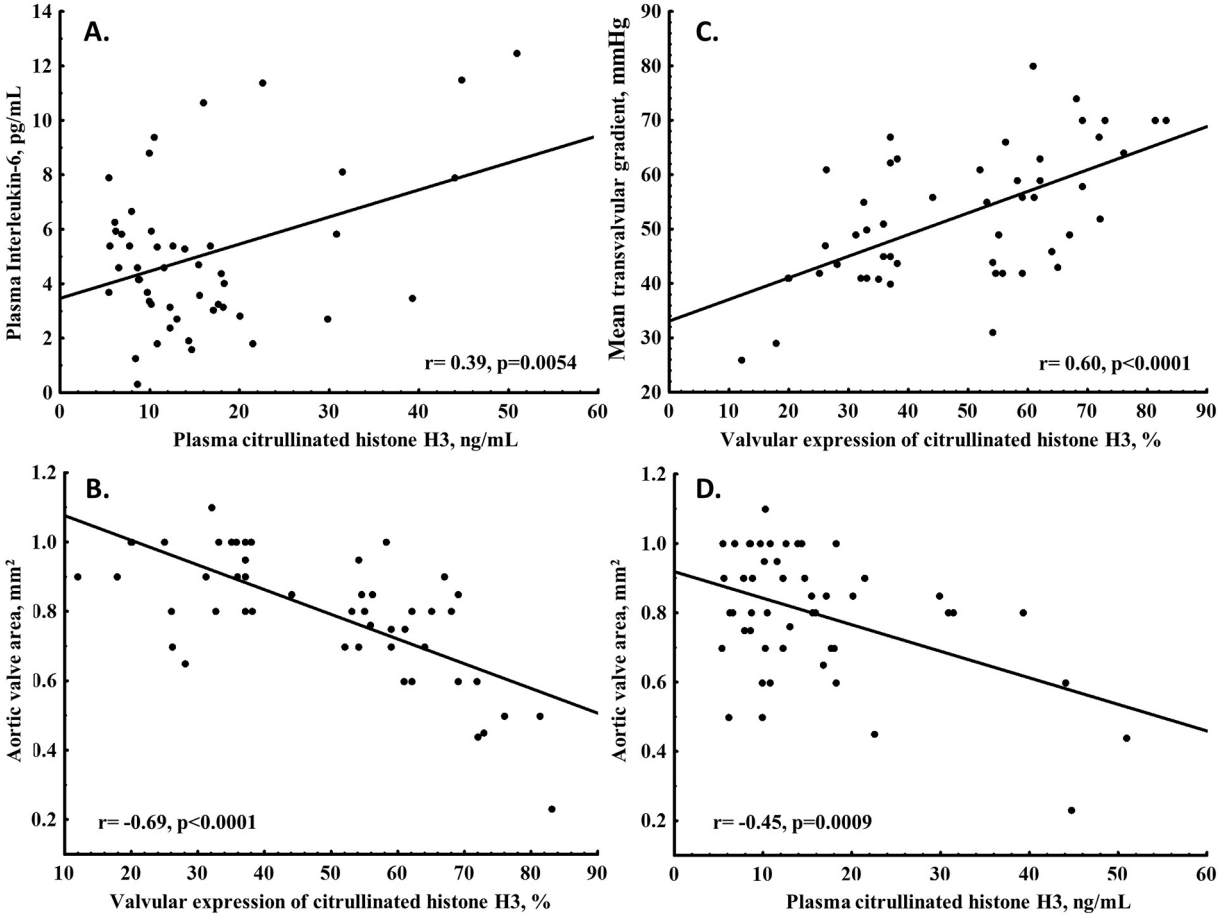

Fig. 3. Pearson's correlation coefficients (*r*) visualized as scatterplots. (A) Plasma interleukin-6 and citrullinated histone H3, (B, C) valvular expression of citrullinated histone H3 and echocardiographic parameters, and (D) plasma citrullinated histone H3 levels and aortic valve area in patients with severe aortic stenosis.

This study has several limitations. The group size was limited and type II errors cannot be avoided. However, the study represents typical patients with advanced AS in clinical practice and it was sufficiently powered to detect intergroup differences. Second, the expression of NET-specific biomarkers was determined semiquantitatively, thus potentially making the estimation less precise. However, all analyses were performed by two independent experienced investigators unaware of the sample origin. Third, the present study is cross-sectional by design and refers to the valves obtained from patients with severe AS, often having hypertension and hypercholesterolemia concerned as confounding factors [12,36]. Thus, our results cannot be directly extrapolated on subjects with less severe AS.

The present study increases the knowledge on the pathophysiology of AS suggesting that NETosis might be involved in AS progression. NETosis is not the factor triggering AS development but rather NETs can, at least partially, contribute to AS progression and vice versa but most likely at advanced stage of the disease. Further studies are needed to validate this intriguing concept.

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Any potential conflicts of interest, including related consultancies, shareholdings and funding grants

The authors report no relationships that could be construed as a conflict of interest.

References

[1] R.L. Osnabrugge, et al., Aortic stenosis in the elderly: disease prevalence and number of candidates for transcatheter aortic valve replacement: a meta-analysis and modeling study, J. Am. Coll. Cardiol. 62 (2013) 100–1012.
[2] K.E. Yutzey, et al., Calcific aortic valve disease: a consensus summary from the Alliance of Investigators on Calcific Aortic Valve Disease, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 2387–2393.
[3] E.R. Mohler 3rd., Are atherosclerotic processes involved in aortic-valve calcification? Lancet 356 (2000) 524–525.
[4] R.V. Freeman, C.M. Otto, Spectrum of calcific aortic valve disease: pathogenesis, disease progression, and treatment strategies, Circulation 111 (2005) 3316–3326.
[5] D. Mohty, et al., Association between plasma LDL particle size, valvular accumulation of oxidized LDL, and inflammation in patients with aortic stenosis, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 187–193.
[6] N.M. Rajamannan, et al., Calcific aortic valve disease: not simply a degenerative process: a review and agenda for research from the National Heart and Lung and Blood Institute Aortic Stenosis Working Group. Executive summary: calcific aortic valve disease – 2011 update, Circulation 124 (2011) 1783–1791.
[7] H. Akahori, T. Tsujino, T. Masuyama, M. Ishihara, Mechanisms of aortic stenosis, J. Cardiol. 71 (2018) 215–220.
[8] C.M. Otto, J. Kuusisto, D.D. Reichenbach, A.M. Gown, K.D. O'Brien, Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies, Circulation 90 (1994) 844–853.
[9] S. Helske, et al., Induction of local angiotensin II-producing systems in stenotic aortic valves, J. Am. Coll. Cardiol. 44 (2004) 1859–1866.
[10] R. Bouchareb, et al., Activated platelets promote an osteogenic programme and the progression of calcific aortic valve stenosis, Eur. Heart J. (2018 Nov 5) https://doi.org/10.1093/eurheartj/ehy696.
[11] J. Natorska, G. Marek, J. Sadowski, A. Undas, Presence of B cells within aortic valves in patients with aortic stenosis: relation to severity of the disease, J. Cardiol. 67 (2016) 80–85.
[12] X. Yu, J. Tan, S.L. Diamond, Hemodynamic force triggers rapid NETosis within sterile thrombotic occlusions, J. Thromb. Haemost. 16 (2018) 316–329.
[13] A.N. Rao, N.M. Kazzaz, J.S. Knight, Do neutrophil extracellular traps contribute to the heightened risk of thrombosis in inflammatory diseases? World J. Cardiol. 7 (2015) 829–842.

[14] J. Natorska, A. Undas, Blood coagulation and fibrinolysis in aortic valve stenosis: links with inflammation and calcification, Thromb. Haemost. 114 (2015) 217–227.
[15] C. Mozzini, U. Garbin, A.M. Fratta Pasini, L. Cominacini, An exploratory look at NETosis in atherosclerosis, Intern. Emerg. Med. 12 (2017) 13–22.
[16] C. Pérez-Sánchez, et al., Diagnostic potential of NETosis-derived products for disease activity, atherosclerosis and therapeutic effectiveness in Rheumatoid Arthritis patients, J. Autoimmun. 82 (2017) 31–40.
[17] V. Brinkmann, et al., Neutrophil extracellular traps kill bacteria, Science. 303 (2004) 1532–1535.
[18] H. Qi, S. Yang, L. Zhang, Neutrophil extracellular traps and endothelial dysfunction in atherosclerosis and thrombosis, Front. Immunol. 8 (928) (2017).
[19] P. Jankowski, et al., Polish Forum for Prevention Guidelines on Smoking: update 2017, Kardiol. Pol. 75 (2017) 409–411.
[20] J. Natorska, et al., Does diabetes accelerate the progression of aortic stenosis through enhanced inflammatory response within aortic valves? Inflammation 35 (2012) 834–840.
[21] Q. Zeng, et al., Cross-talk between the Toll-like receptor 4 and Notch1 pathways augments the inflammatory response in the interstitial cells of stenotic human aortic valves, Circulation 126 (2012) S222–S230.
[22] D. El Husseini, et al., P2Y2 receptor represses IL-6 expression by valve interstitial cells through Akt: implication for calcific aortic valve disease, J. Mol. Cell. Cardiol. 72 (2014) 146–156.
[23] A. Caudrillier, et al., Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury, J. Clin. Invest. 122 (2012) 2661–2671.
[24] J.I. Borissoff, et al., Elevated levels of circulating DNA and chromatin are independently associated with coronary atherosclerosis and a prothrombotic state, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 2032–2040.
[25] V. Delgado-Rizo, et al., Neutrophil extracellular traps and its implications in inflammation: an overview, Front. Immunol. 8 (81) (2017).
[26] R.T. Megens, et al., Presence of luminal neutrophil extracellular traps in atherosclerosis, Thromb. Haemost. 107 (2012) 597–598.
[27] N.A. Aulik, K.M. Hellenbrand, C.J. Czuprynski, Mannheimia haemolytica and its leukotoxin cause macrophage extracellular trap formation by bovine macrophages, Infect. Immun. 80 (2012) 1923–1933.
[28] P. Liu, et al., Escherichia coli and Candida albicans induced macrophage extracellular trap-like structures with limited microbicidal activity, PLoS One 9 (2014), e90042.
[29] S. Bonne-Année, et al., Extracellular traps are associated with human and mouse neutrophil and macrophage mediated killing of larval Strongyloides stercoralis, Microbes Infect. 16 (2014) 502–511.
[30] K.W. Wong, W.R. Jacob Jr., Mycobacterium tuberculosis exploits human interferon γ to stimulate macrophage extracellular trap formation and necrosis, J. Infect. Dis. 208 (2013) 109–119.
[31] K. Kambas, I. Mitroulis, K. Ritis, The emerging role of neutrophils in thrombosis-the journey of TF through NETs, Front. Immunol. 3 (385) (2012).
[32] K. Kambas, et al., Tissue factor expression in neutrophil extracellular traps and neutrophil derived microparticles in antineutrophil cytoplasmic antibody associated vasculitis may promote thromboinflammation and the thrombophilic state associated with the disease, Ann. Rheum. Dis. 73 (2014) 1854–1863.
[33] P. Kapusta, et al., Factor XIII expression within aortic valves and its plasma activity in patients with aortic stenosis: association with severity of disease, Thromb. Haemost. 108 (2012) 1172–1179.
[34] J. Natorska, et al., Fibrin presence within aortic valves in patients with aortic stenosis: association with in vivo thrombin generation and fibrin clot properties, Thromb. Haemost. 105 (2011) 254–260.
[35] J. Breyne, et al., Atherosclerotic-like process in aortic stenosis: activation of the tissue factor-thrombin pathway and potential role through osteopontin alteration, Atherosclerosis. 213 (2010) 369–376.
[36] A.R. Tall, M. Westerterp, Inflammasomes, neutrophil extracellular traps, and cholesterol, J. Lipid Res. 60 (4) (2019) 721–727.