ORIGINAL ARTICLE

Influence of osteoclasts and osteoprotegerin on the mode of calcific degeneration of aortic valves

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KEY WORDS

aortic stenosis, calcification, M2 macrophages, osteoclasts, osteoprotegerin

ABSTRACT

INTRODUCTION Calcific aortic valve disease is associated with inflammation and calcification, thus the osteoprotegerin (OPG), receptor activator of nuclear factor κB (RANK) and its ligand (RANKL) system involved in osteoclastogenesis and inflammation may play a significant role in valve degeneration.

OBJECTIVES The aim of this study was to assess whether circulating OPG, sRANKL, and other bone metabolism markers can predict the presence of osteoclasts in stenotic valves and to evaluate their impact on the mode of degeneration.

PATIENTS AND METHODS The study involved 60 patients with aortic stenosis who underwent valve replacement surgery and subsequently were divided into 2 groups: osteoclastic (n = 12) and nonosteoclastic (n = 48), according to the presence or absence of intravalvular osteoclasts. Before the surgery, we measured serum levels of OPG, sRANKL, osteocalcin, osteopontin, tumor necrosis factor α (TNF-α), interleukin (IL) 1β, and IL-6. Immunohistochemistry and morphometry were used to determine the extent of valve calcification, lipid accumulation, neovascularization, and the number and phenotype of macrophages.

RESULTS Compared with the nonosteoclastic group, patients with intravalvular osteoclasts had lower levels of OPG ($P = 0.0006$) and TNF-α ($P = 0.02$) and less frequently had diabetes ($P = 0.04$). Their valves showed higher incidence of ossification ($P = 0.002$), higher total ($P = 0.008$) and M2 macrophage counts ($P = 0.0002$), increased neovascularization ($P = 0.003$), and lower accumulation of lipids ($P = 0.04$). They also showed a negative correlation between valve calcification and age ($r = –0.79$, $P = 0.002$), which was not observed in patients without osteoclasts. In a multivariate analysis, low circulating OPG levels and the absence of diabetes were predictors of intravalvular osteoclastic differentiation.

CONCLUSIONS The presence of osteoclasts in stenotic valves associated with low circulating OPG levels and an enhanced proportion of M2 macrophages can represent a variant of calcific aortic valve disease with a specifically regulated calcification process.

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Received: January 28, 2016.
Revision accepted: February 18, 2016.
Published online: March 22, 2016.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2016; 126 (3): 149-158
doi: 10.20452/pamw.3326

INTRODUCTION Calcific aortic valve disease, the main cause of valve replacement in elderly patients, is regarded as an active process akin to atherosclerosis, involving mechanisms that can undergo modifications with disease progression.[1,2] The process is associated with the appearance of cells with osteoblastic and osteoclastic features.[1,3-5] The majority of studies focused on osteoblastic differentiation of valvular or vascular cells, as well as those describing osteoclastic differentiation, concerned a broader range of osteoclastic-like cells, that is, both multi- and mononuclear cells expressing osteoclastic marker (tartrate-resistant acid phosphatase [TRAP]).[3,5]

TRAP-positive cells are not, however, a homogeneous population. TRAP is indeed characteristic

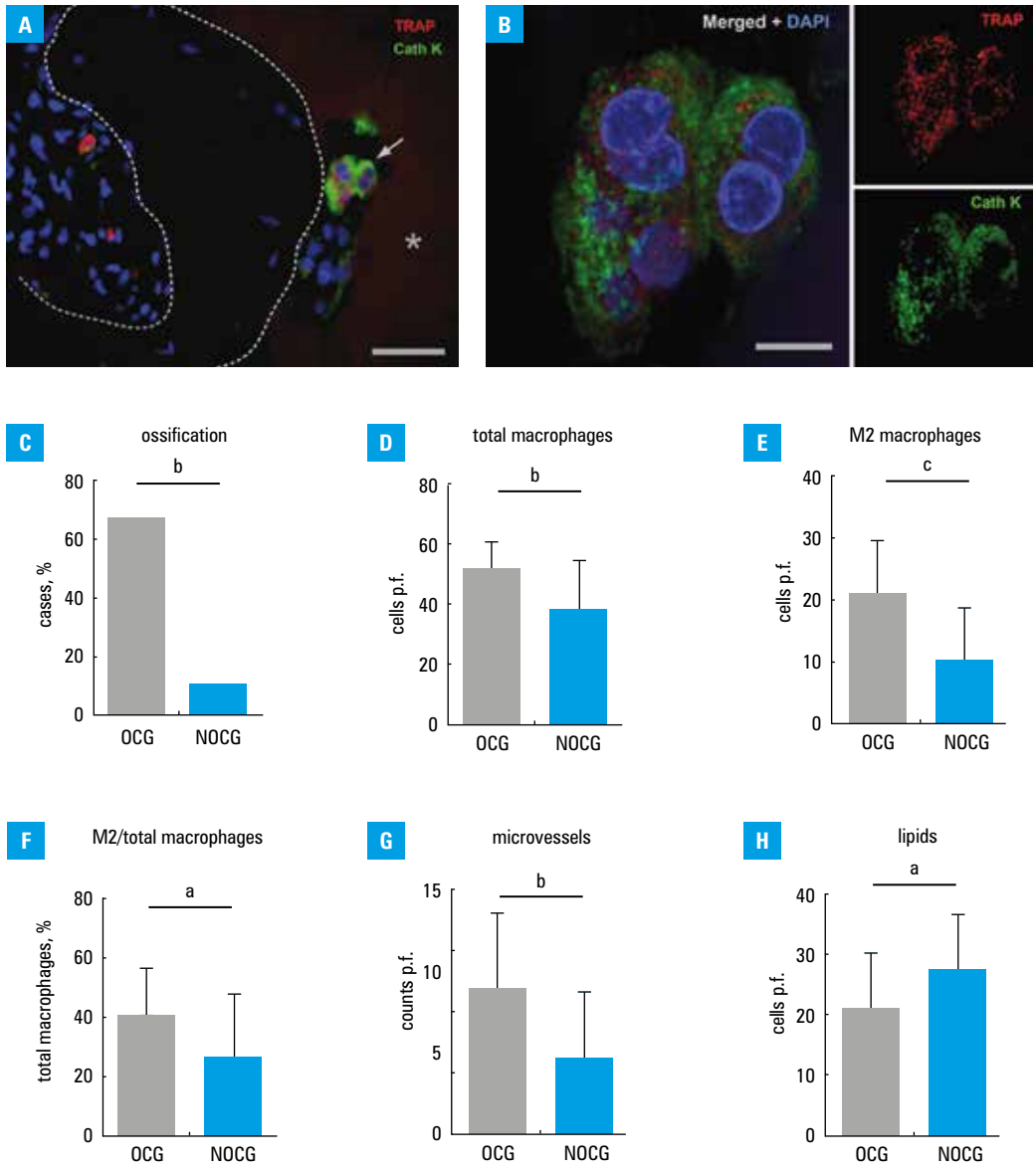

FIGURE 1 Characteristic features of valve tissue remodeling associated with the presence of osteoclasts; **A** – tartrate-resistant acid phosphatase (TRAP)-positive / cathepsin K (Cath K)-positive osteoclasts (arrow) located between the ossified area (dotted line) and dystrophic calcification (asterisk); **B** – confocal microscopic image of osteoclasts shown in **A**; cell nuclei stained with DAPI; bars, 50 μm (**A**) and 10 μm (**B**); **C–H** – differences between osteoclastic (OCG) and nonosteoclastic (NOCG) groups: patients with ossification, number of total macrophages, M2 macrophages and their proportions, microvessel density and lipid area; **a** $P < 0.05$; **b** $P < 0.01$; **c** $P < 0.001$

for cells undergoing osteoclastic differentiation, but can also be expressed by other cells belonging to the monocyte and macrophage lineage,[6] so mononuclear TRAP-positive cells only partly represent early differentiating osteoclasts. That is why, in the present study, we analyzed only fully differentiated, multinuclear TRAP-positive osteoclasts.

Differentiation and recruitment of osteoclasts in skeletal tissues is mainly controlled by osteoprotegerin (OPG), receptor activator of nuclear factor κB (RANK), and its ligand (RANKL) signaling system. By integrating the regulation of bone tissue metabolism as well as immune and inflammatory processes, the OPG/RANK/RANKL system can be an important player in calcific aortic valve disease, since bone-associated cells and matrix components as well as immune cells and inflammatory mediators are involved in the disease. It was shown that serum OPG levels tended to increase with age in healthy individuals and were elevated in patients with calcific aortic valve disease[5,7-9]; moreover, OPG revealed altered expression in stenotic valves.[10] Recent publications have also suggested that circulating biomarkers of bone turnover are associated with the severity of stenosis and vascular calcification.[3,11] The involvement of OPG in cardiovascular disease is now widely discussed, with an emphasis on its dichotomous (proatherogenic and antiatherogenic) role.[12,13]

Both local and systemic inflammation can drive cardiovascular calcification.[14] Proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), increase the levels of circulating OPG via the activation of endothelial cells.[15] On the other hand,

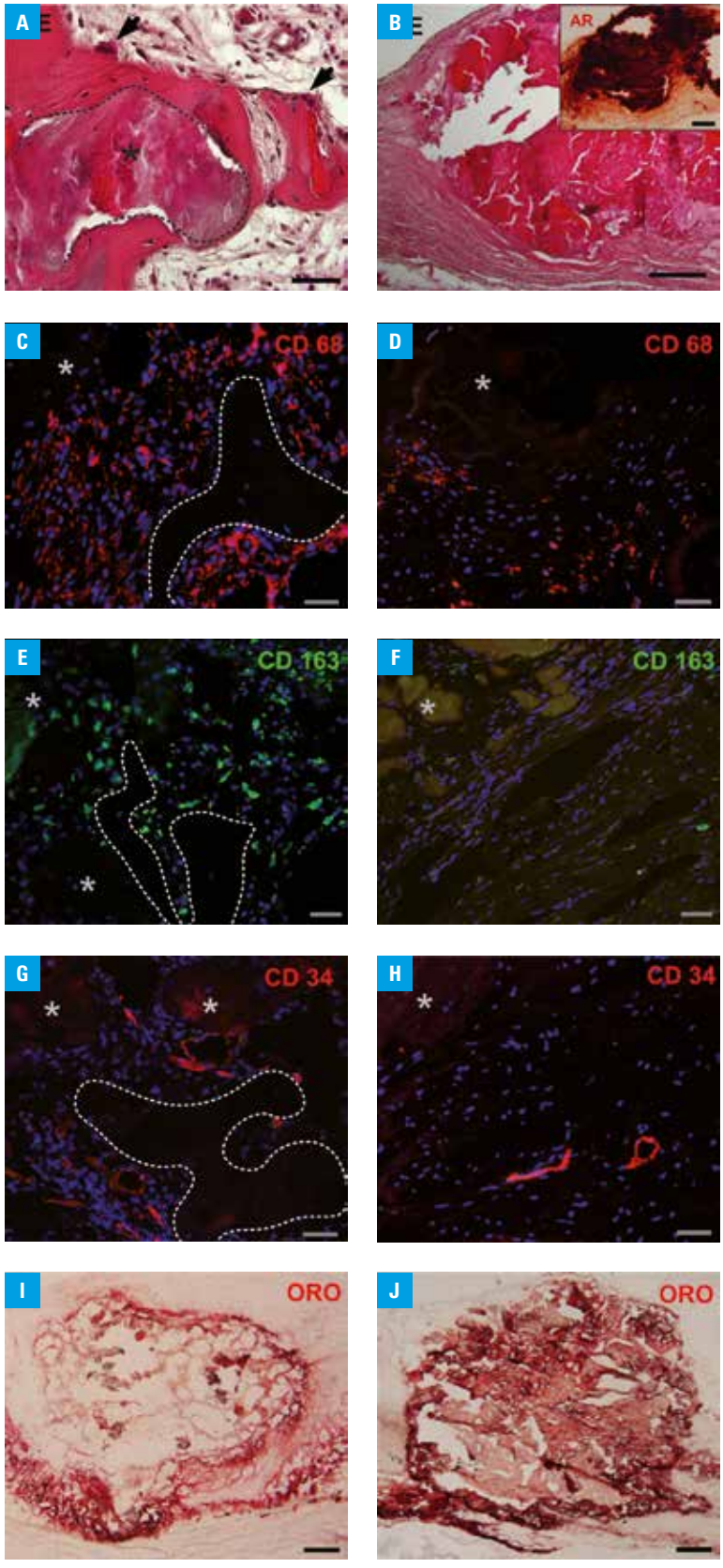

FIGURE 2 Representative micrographs illustrating differences between the valves of osteoclastic group (left row) and nonosteoclastic group (right row) in calcification (**A** and **B**), total CD68-positive and M2 CD163-positive macrophage infiltration (**C**, **D** and **E**, **F**), neovascularization (**G** and **H**), and lipid content (**I** and **J**). Note the presence of osteoclasts (arrows in **A**) and areas of ossification (outside of dotted line in **A**) above the dystrophic form of calcification (asterisk), the only calcification form seen in nonosteoclastic group (**B**), also visualized by alizarin red (AR; insert in **B**). Asterisks in **C**–**H** mark dystrophic calcification; dotted line in **C**, **E**, **G** marks ossification; bars, 500 µm (**A**, **B**, **B** [insert], **I**, **J**) and 50 µm (**C**, **D**, **E**, **F**, **G**, **H**).

cytokines such as interleukin (IL)-1, IL-6, and TNF-α have been demonstrated to show osteoclastogenic and osteogenic activity.[16-18] Macrophages are the largest population of inflammatory cells observed in calcific aortic valve disease. They reveal phenotypes with "pro-" and "anti-inflammatory" activity, classified as M1 and M2, respectively.[19] Such phenotypic macrophage heterogeneity has been well documented in atherosclerotic lesions,[20] but much less is known on their plasticity in calcific aortic valve disease. Therefore, the aim of this study was to assess whether circulating bone metabolism and inflammatory markers can predict the presence of fully differentiated osteoclasts in stenotic valves, and whether their occurrence can be related to specific histomorphological characteristics of valve degeneration and to the course of valve calcification.

PATIENTS AND METHODS

Characteristics of patients and tissue material Stenotic aortic valves (n = 60) were obtained from patients undergoing routine valve replacement surgery. Patients with cancer, autoimmune disorders, end-stage renal failure, or ongoing infective process were excluded from the study. Only valves without signs of infective endocarditis or rheumatic heart disease were examined. Data concerning demographics and cardiovascular risk factors were collected using a standard questionnaire and previous medical documentation.

Prior to surgery, all patients underwent transthoracic echocardiography and routine laboratory blood tests. Additional serum samples for the measurement of bone metabolism and inflammatory biomarkers were stored at –80°C until use. The study protocol was approved by the Jagiellonian University Bioethical Committee, and all patients signed an informed consent form.

Assessment of bone metabolism and inflammatory biomarkers The levels of OPG, osteopontin, osteocalcin, TNF-α, IL-1β, IL-6, and soluble RANKL (sRANKL) were measured in serum samples by a multiplex method and enzyme-linked immunosorbent assay (sRANKL). For details, see the Supplementary material online.

Assessment of valve calcification area Calcification area was measured morphometrically in the valve cusps examined under Stemi 2000C stereomicroscope (Zeiss, Germany) with the use of AnalySIS-FIVE® (Soft Imaging System GmbH, Münster, Germany) image analysis system and expressed as percentage of the total cusp area.

Histochemistry and immunohistochemistry Valve sections were stained routinely with hematoxylin and eosin, with Oil red O to reveal lipids, and with Alizarin red to visualize calcified areas. Osteoclasts, macrophages, and blood vessels were visualized by immunofluorescent detection of cell-specific antigens: TRAP and cathepsin K double staining for osteoclasts, CD68 for total

TABLE 1 Comparison of clinical and biochemical parameters in osteoclastic and nonosteoclastic groups

Parameter	Osteoclastic group	Nonosteoclastic group	*P* value
demographic and clinical parameters			
age, y	63.08 ±12.72	67.94 ±8.74	0.1
male sex	8 (67)	26 (54)	0.5
aortic mean gradient, mmHg	52.42 ±15.13	54.17 ±16.44	0.7
aortic peak gradient, mmHg	85.17 ±27.22	87.33 ±23.72	0.8
AVA, cm^2	0.82 ±0.17	0.77 ±0.2	0.5
bicuspid aortic valve	8 (67)	23 (48)	0.3
aortic valve insufficiency	4 (33)	15 (31)	1.0
coronary artery disease	3 (25)	20 (42)	0.3
history of myocardial infarction	2 (17)	8 (17)	1.0
atrial fibrillation	2 (17)	9 (19)	1.0
hypertension	10 (83)	39 (81)	1.0
BMI, kg/m^2	30.03 ±5.95	28.95 ±4.49	0.5
hyperlipidemia	8 (67)	40 (83)	0.2
diabetes	1 (8)	21 (44)	0.04
chronic renal failure	3 (25)	5 (10)	0.2
statins	5 (42)	24 (50)	0.8
biochemical markers			
OPG, ng/ml	0.19 (0.15–0.29)	0.38 (0.25–0.54)	0.0006
sRANKL, pmol/l	135.4 (85.72–231.0)	118.5 (72.37–155.0)	0.4
OPG/sRANKL, ×100	0.15 (0.11–0.26)	0.35 (0.19–0.51)	0.003
OPN, ng/ml	6.71 (4.69–1.49)	10.31 (4.22–15.07)	0.4
OCN, ng/ml	5.21 (2.93–7.99)	5.78 (2.96–9.29)	0.5
TNF-α, pg/ml	1.86 (1.8–3.42)	3.58 (2.17–6.32)	0.02
IL-1β, pg/ml	0.24 (0.17–0.28)	0.24 (0.17–0.33)	0.6
IL-6, pg/ml	1.17 (0.57–1.67)	1.37 (0.82–5.01)	0.09
calcium, mmol/l	2.29 ±0.06	2.31 ±0.08	0.4
phosphorus, mmol/l	1.24 ±0.13	1.24 ±0.21	1.0

Data are presented as means ± standard deviation, medians (lower and upper quartiles), or number (percentage) of patients.

Abbreviations: AVA, aortic valve area; BMI, body mass index; IL-1β, interleukin 1β; IL-6, interleukin 6; OCN, osteocalcin; OPG, osteoprotegerin; OPN, osteopontin; sRANKL, soluble receptor activator of nuclear factor κB ligand; TNF-α, tumor necrosis factor α

macrophages, CD163 for M2 macrophages, and CD34 for microvessels. For details, see the Supplementary material online.

Microscopy, confocal microscopy, and morphometry Tissue sections were examined under a laser scanning (confocal) fluorescence microscope (FluoView FV1200, Olympus, Tokyo, Japan) and an Olympus BX50 bright field/fluorescence microscope equipped with a computer-assisted image analysis system. Macrophages (total and M2) were expressed as cell number per high power field; microvessels, as the number of profiles per high power field; and the area occupied by lipids, as the percentage of the total cusp section area. For details, see the Supplementary material online.

Statistical analysis Statistical analyses (see the Supplementary material online) were performed using Statgraphics Centurion XVI (StatPoint Technologies INC, Warrenton, Virginia, United States) software. All tests were 2-tailed with a *P* value of less than 0.05 considered as statistically significant.

RESULTS **Identification of osteoclasts** Large multinucleated TRAP+/cathepsin K+ cells, regarded as differentiated osteoclasts and located in calcified or ossified areas (FIGURES 1 and 2), were found in the valves of 12 patients. These patients and their valves (classified as the osteoclastic group) were compared with patients whose valves did not contain intravalvular osteoclasts (nonosteoclastic group, n = 48).

Characteristics of osteoclastogenic milieu The valves with osteoclasts more frequently showed osteogenic metaplasia, higher number of total and M2 macrophages, higher proportion of M2/total macrophages, higher microvessel density, and lower valve area occupied by lipids (FIGURES 1 and 2). There was no significant

TABLE 2 Final model of stepwise multivariate logistic regression for the occurrence of osteoclasts

	β	SE	*P* value
OPG levels (low)	2.745	1.115	0.017
diabetes	–2.277	1.130	0.014

The model was adjusted for age, sex, and aortic valve morphology (bicuspid). TNF-α and IL-6 were tested as potential covariates.

Abbreviations: SE, standard error; others, see TABLE 1

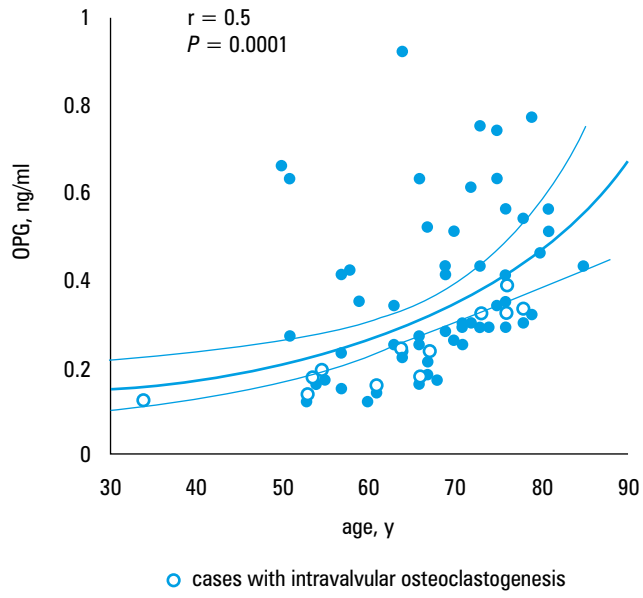

FIGURE 3 Correlation between osteoprotegerin (OPG) and age in the whole study population; nonlinear fitting (thick line) and 95% confidence intervals for the fitting (thin lines)

difference in the calcification area (45.21 ±14.09 and 41.82 ±16.97 for osteoclastic and nonosteoclastic group, respectively, $P = 0.3$).

Clinical and biochemical factors associated with osteoclastogenesis A univariate comparison of clinical and biochemical parameters revealed that patients from the osteoclastic group had lower serum OPG levels, OPG/sRANKL ratio, TNF-α levels, and incidence of diabetes than patients from the nonosteoclastic group (TABLE 1). A stepwise multivariate logistic regression analysis including the above variables and IL-6, which in a univariate analysis approached significance ($P < 0.1$), showed that low OPG levels and diabetes were positive and negative predictors of the presence of osteoclasts, respectively (TABLE 2). An independent role of diabetes was stressed by the fact that in the group of diabetic patients with the OPG level "permissive" for osteoclastogenesis (ie, not exceeding the highest value found in the osteoclastic group), almost all patients (11 of 12) had no osteoclasts in their valves ($P = 0.03$).

Moreover, our analysis showed that OPG levels strongly correlated with markers of inflammatory status (TNF-α and IL-6, both, $P < 0.0001$; Supplementary material online, *Table S1*), and that diabetic patients (n = 22) had significantly higher body mass index and TNF-α concentrations than nondiabetic ones (32.08 ±4.99 kg/m^2 vs 27.6 ±3.93 kg/m^2, $P = 0.0009$, for body mass index, and 3.9 pg/ml [3.09–6.32] vs 2.9 pg/ml [1.82–5.06], $P = 0.03$, for TNF-α concentrations).

Correlation between osteoprotegerin levels and age in stenotic patients: implications for osteoclastogenesis In the whole study group, circulating OPG levels were significantly correlated with age ($r = 0.56$; $P < 0.0001$). A nonlinear regression model showed a relatively slow increase in OPG levels in younger patients and a markedly enhanced increase in older individuals (FIGURE 3). It is of note that all cases with osteoclasts were located below the trend line, as depicted in the graph.

We also checked whether the occurrence of osteoclasts might interfere with the correlation of OPG with age. In the osteoclastic group, OPG strongly correlated with age ($r = 0.9$; $P = 0.0001$), while in the nonosteoclastic group, the correlation was weak ($r = 0.39$; $P = 0.01$) and OPG values were much more variable (FIGURE 4), suggesting the effect of other factors on the OPG level.

Effect of osteoclasts and osteoprotegerin on the association between calcification and age There was no correlation between valve calcification area and patients' age in the whole study group ($P = 0.2$). However, the presence of intravalvular osteoclasts substantially altered the association between calcification and age, since the osteoclastic group revealed a strong negative correlation of calcification with age ($r = –0.79$; $P = 0.002$), not observed in the nonosteoclastic group ($r = 0.09$; $P = 0.5$) (FIGURE 4). We also examined whether the calcification–age relationship was influenced by the OPG level as a predictor of osteoclastogenesis. Indeed, in patients with low OPG levels, permissive for osteoclastogenesis, calcification negatively correlated with age ($r = –0.5$; $P = 0.002$), while patients with high OPG levels showed a positive correlation with borderline significance ($r = 0.41$; $P = 0.05$) (FIGURE 5).

DISCUSSION We have shown for the first time that low circulating OPG levels predict the occurrence of fully differentiated osteoclasts and that osteoclast-containing valves differ from those without osteoclasts. Another important finding of our study is that the occurrence of osteoclasts in patients with calcific aortic valve disease is related to general and local inflammatory status, as manifested by inflammatory cytokine levels and their associations with OPG, as well as by enhanced absolute and relative count of M2 macrophages. OPG seems to play a pivotal role here.

To our knowledge, the link between M2 macrophages associated with tissue repair and maintenance of its integrity[21] and osteoclastic differentiation in stenotic aortic valves has been reported for the first time. This link was postulated in patients with periprosthetic osteolysis, in whom M2 macrophages coincided with increased

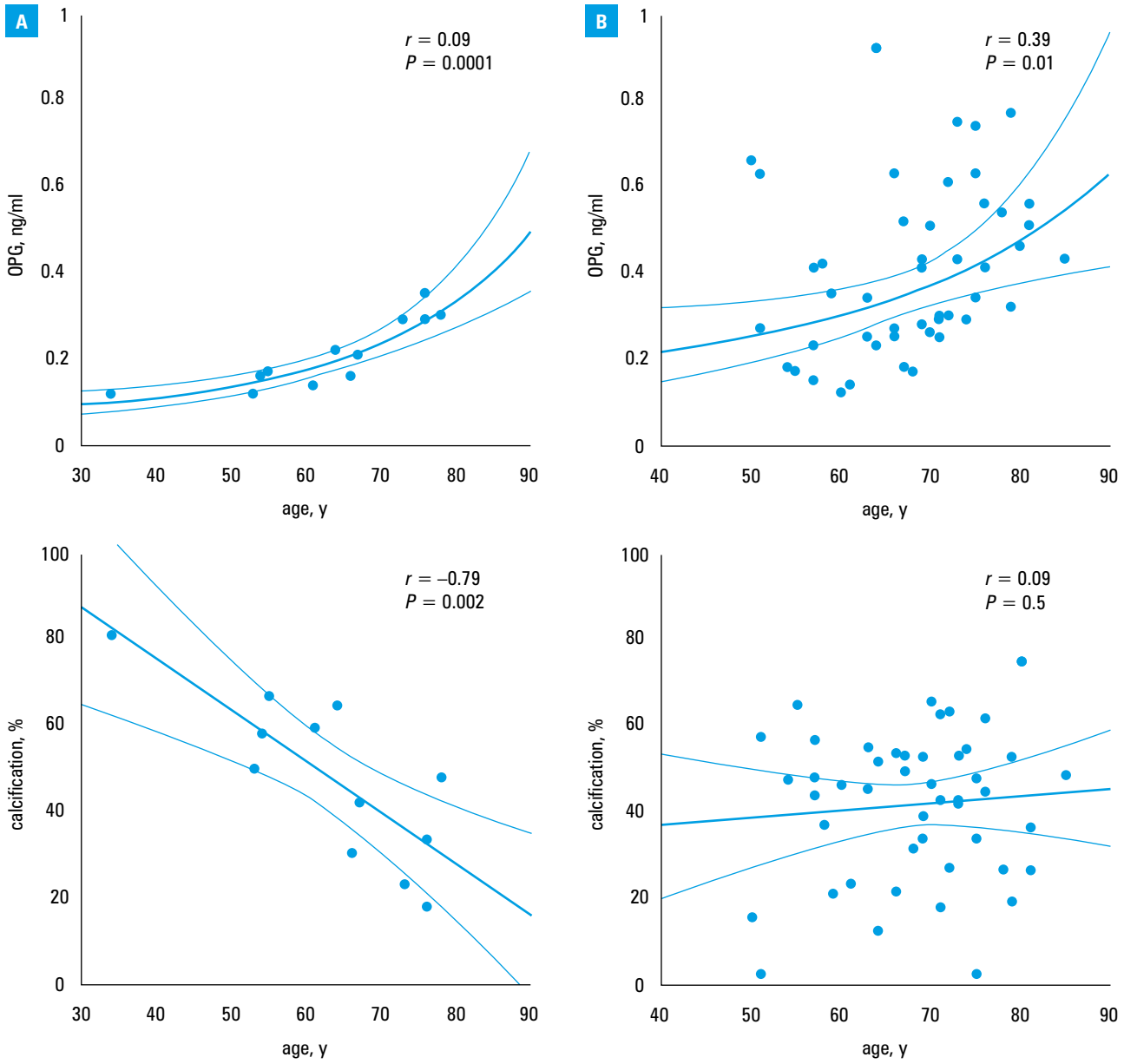

FIGURE 4 Correlation of osteoprotegerin (OPG) with age and valve calcification with respect to the presence of osteoclasts in the osteoclastic group (**A**) and nonosteoclastic group (**B**); linear or nonlinear fittings (thick line), and 95% confidence intervals for the fittings (thin lines)

recruitment and maturation of osteoclast precursors.[22] Recently published reports have supported the association of M2 polarization with the absence of OPG[23] and younger age[24] in mice models. The increased neovascularization observed in the osteoclastic group can facilitate recruitment of macrophage and osteoclast precursors from blood to degenerating valves.

The negative correlation between diabetes and the presence of osteoclastic aortic valve is also a novel finding. Type 2 diabetes mellitus has recently emerged as a significant metabolic risk factor for calcific aortic valve disease and accelerated degeneration of bioprosthetic valves.[25,26] O'Sullivan et al[27] showed increased serum OPG levels in diabetic patients. Our results suggest the impact of diabetes on the mode of calcific valve remodeling irrespectively of the OPG level.

It remains controversial how the presence of osteoclasts could influence the local calcification process in the valve. The regulatory role of osteoclastic-like cells in the maintenance of mineral homeostasis within the atherosclerotic arterial wall was postulated by Doherty et al.[28] They suggested that vascular calcium deposition may be affected by the presence and activity of osteoclast-like cells, which are able to inhibit or even revert the ongoing calcification. However, mouse model studies indicated a procalcific influence of osteoclast-like cells on the cardiovascular system.[29] Moreover, experimental data suggest that the activity of osteoclasts is critical for effective osteogenesis.[30]

Recently, Nagy et al[3] reported that systemic levels and local mRNA expression of TRAP (regarded as osteoclast-associated marker) positively correlated with selected echocardiographic parameters of stenosis severity. We did not observe such a correlation, but in contrast to Nagy et al, we analyzed cases showing the presence of

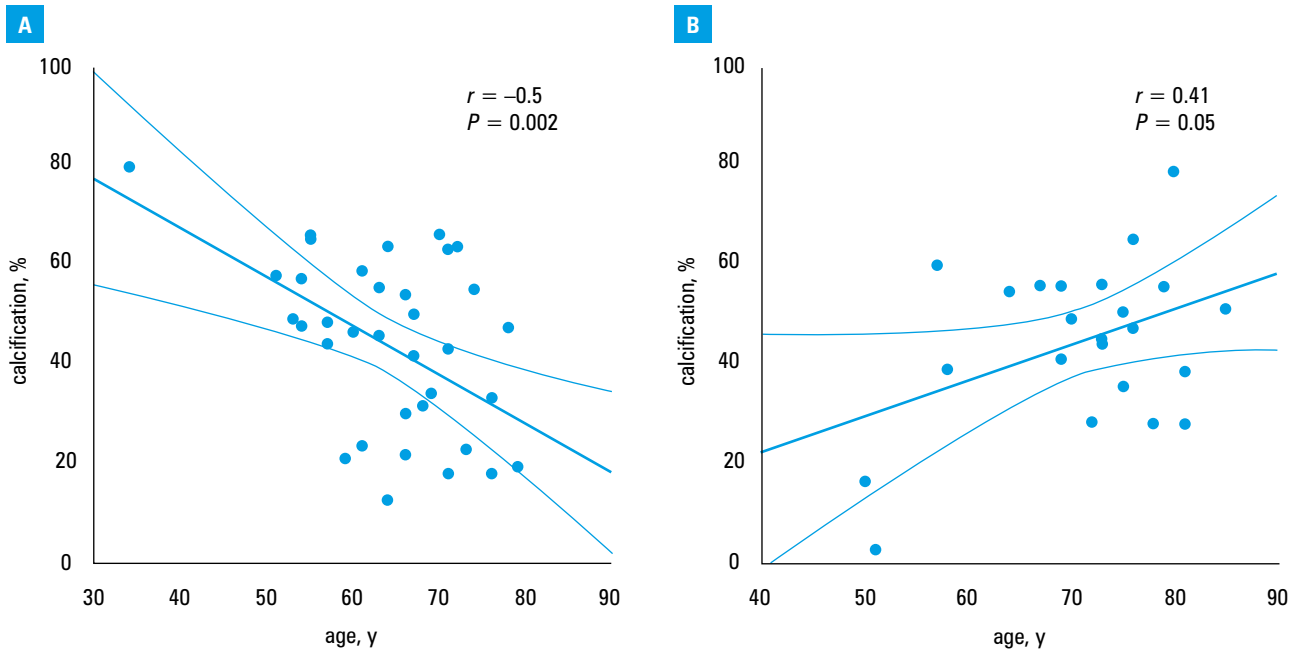

FIGURE 5 Correlation between calcification and age with respect to serum osteoprotegerin (OPG) levels: **A** – low OPG level (not exceeding the highest value found in the osteoclastic group); **B** – high OPG level (exceeding values found in the osteoclastic group); linear fittings (thick line) and 95% confidence intervals for the fittings (thin lines).

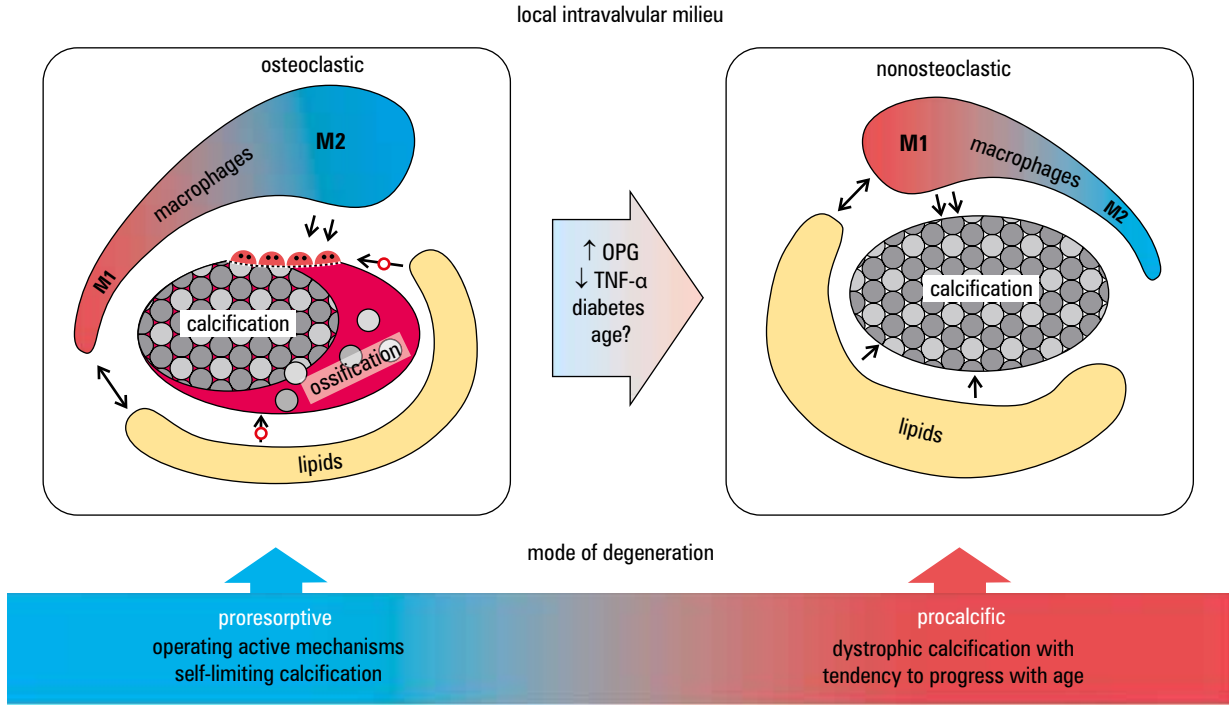

FIGURE 6 Modes of aortic valve degeneration reflecting the modifications in the local milieu. Occurrence of osteoclasts associated with low serum osteoprotegerin (OPG) level and local milieu characterized by enhanced absolute and relative number of M2 macrophages, ossification, microvessels, and lower accumulation of lipids promote "proresorptive" mode of valve degeneration, in which osteoclastic milieu slows down the spread of calcification but facilitates local ossification. Elevation of OPG concentrations (preventing osteoclast formation) results in progressive calcification of valve tissue ("procalcific" mode). This mode could also be switched on by general inflammation or diabetes and is probably more prevalent in aged patients due to "naturally" increasing OPG levels with age. Arrows indicate possible positive or negative (red circle) influences.
Abbreviations: TNF-α, tumor necrosis factor α

fully differentiated multinucleated osteoclasts. TRAP is also expressed by macrophages,[6] which are more common in stenotic valves than mature osteoclasts.

The association of osteoclasts with ossification suggests an intimate coupling of these cells with the active mode of calcific remodeling, which results in intravalvular bone formation. Thus, the occurrence of osteoclasts seems to facilitate a

switch of the calcification mode towards an active "osteogenic program". Moreover, we found a negative correlation of calcification with patients' age in the osteoclastic group, which might suggest that the activity of osteoclasts could limit the calcification area or that the entire milieu characterized by the presence of osteoclasts can promote processes oriented towards restriction of the age-dependent progression of calcification. On the other hand, since pathologically altered valve areas rich in lipids reveal much higher calcium content than the areas not accumulating lipids,[31] the higher accumulation of lipids in the nonosteoclastic group might contribute to the development of less modifiable milieu and, consequently, calcification progression with age.

The correlation of OPG with age, characterized by a more notable increase and higher variability of OPG in older individuals, is in line with other reports.[7,8] This finding can have important implications. Considering that all cases with osteoclasts were located below the OPG trend line increasing with age, it is possible that not the absolute serum OPG concentration but rather its level relative to age either permits or inhibits osteoclastogenesis in the valves. Cases with OPG levels permissive for osteoclastogenesis (low for age) are associated with a negative correlation between calcification and age, resulting in lower calcification area in patients at advanced age. This might indicate that relatively low OPG levels and the resulting local proosteoclastic milieu may constitute a specific "proresorptive" variant of valve degeneration (**FIGURE 6**), contrary to the more frequent "procalcific" variant associated with elevated OPG levels and a positive correlation between calcification and age, which corresponds with the findings from human studies.[32] To establish whether any of these variants is more beneficial for patients is outside the scope of this study. Interestingly, there are data indicating an association of OPG with left ventricular and atrial remodeling and suggesting a more favorable outcome in postoperative follow-up in stenotic patients with lower preoperative OPG levels.[33]

Our results suggest a variable role of OPG in calcific aortic valve disease, which makes it difficult to design a unified therapeutic strategy targeting the OPG/RANK/RANKL system that would be effective in a broad spectrum of patients with calcific aortic valve disease. Indeed, the effectiveness of denosumab, an anti-RANKL antibody mimicking the action of OPG, has not been confirmed in clinical trials.[34] Also, data concerning the effect of statins, which reduce not only lipid activity but also the levels of OPG and sRANKL, are still inconclusive.[2,35]

The limitation of our study is the observational design, which enables us to demonstrate associations but not causal relationships.

In conclusion, we demonstrated that the presence of differentiated osteoclasts in calcified stenotic aortic valves associated with a relatively low concentration of circulating OPG and enhanced proportion of intravalvular M2 macrophages are the hallmarks of the local milieu with potency to limit calcification. Therefore, serum OPG levels seem to be a promising candidate for a marker that could indicate the prevalent mode of valvular remodeling.

Contribution statement GJL conceived the idea for the study. GJL, UC, and JAL contributed to the design of the research. GJL, UC, AL, JN, BK, and JS were involved in data acquisition. GJL, UC, EJ-G and AL analyzed the data. GJL and JAL drafted the manuscript, and all authors revised it critically for important intellectual content and approved the final version.

Acknowledgments This work was supported by statutory grants (K/ZDS/000987 and K/ZDS/003823, to GJL) from the Jagiellonian University Medical College.

Supplementary material online Supplementary material online is available with the online version of the article at www.pamw.pl.

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ARTYKUŁ ORYGINALNY

Wpływ osteoklastów i osteoprotegeryny na sposób degeneracji wapniowej zastawki aortalnej

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SŁOWA KLUCZOWE

kalcyfikacja, makrofagi M2, osteoklasty, osteoprotegeryna, stenoza aortalna

STRESZCZENIE

WPROWADZENIE Choroba kalcyfikacyjna zastawki aortalnej jest związana z procesem zapalnym i wapnieniem, dlatego układ osteoprotegeryna (OPG), aktywator receptora jądrowego czynnika κB (RANK) i jego ligand (RANKL) związany z osteoklastogenezą i zapaleniem może odgrywać istotną rolę w degeneracji zastawki.

CELE Celem badania była ocena, czy krążące OPG, sRANKL i inne markery metabolizmu kostnego mogą być predyktorami obecności osteoklastów w zastawce stenotycznej oraz ocena ich wpływu na formę degeneracji.

PACJENCI I METODY Do badania włączono 60 pacjentów ze stenozą aortalną, u których dokonano chirurgicznej wymiany zastawki aortalnej i następnie podzielono na 2 grupy: osteoklastyczną (n = 12) i nieosteoklastyczną (n = 48), zależnie od obecności osteoklastów lub ich braku w usuniętych zastawkach. Przed zabiegiem zmierzono poziom OPG, sRANKL, osteokalcyny, osteopontyny, czynnika martwicy guza α (*tumor necrosis factor α* – TNF-α), interleukiny (IL) 1b oraz IL-6 w surowicy pacjentów. W ocenie rozległości zwapnień w zastawkach, akumulacji lipidów, neowaskularyzacji oraz liczby i fenotypu makrofagów stosowano metody immunohistochemiczne i morfometryczne.

WYNIKI Pacjenci z osteoklastami w zastawce, w porównaniu z chorymi z grupy nieosteoklastycznej, cechowali się niższym poziomem OPG (p = 0,0006), TNF-α (p = 0,02) i rzadziej chorowali na cukrzycę (p = 0,04). W ich zastawkach częściej obserwowano występowanie metaplazji kostnej (p = 0,002), liczniejsza była zarówno całkowita populacja (p = 0,008), jak i typ M2 (p = 0,0002) makrofagów, nasilone tworzenie naczyń (p = 0,003) i mniejsze nagromadzenie lipidów (p = 0,04). U pacjentów tych wykazano również negatywną korelację między kalcyfikacją a wiekiem (r = –0,79; p = 0,002), czego nie obserwowano u pacjentów bez osteoklastów. W analizie wieloczynnikowej niski poziom krążącej OPG i brak cukrzycy były predyktorami śródzastawkowego powstawania osteoklastów.

WNIOSKI Obecność osteoklastów w zastawce stenotycznej związana z niskim poziomem krążącej OPG i większą proporcją makrofagów M2 w zastawce może stanowić wariant choroby kalcyfikacyjnej zastawki aortalnej ze szczególną regulacją procesu wapnienia.

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Praca wpłynęła: 28.01.2016.
Przyjęta do druku: 18.02.2016.
Publikacja *online*: 22.03.2016.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2016; 126 (3): 149-158
doi: 10.20452/pamw.3326