ORIGINAL ARTICLE

Potential pathogenic role of soluble receptor activator of nuclear factor-κB ligand and osteoprotegerin in patients with pulmonary arterial hypertension

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KEY WORDS

osteoprotegerin, prognosis, pulmonary arterial hypertension, sRANKL

ABSTRACT

INTRODUCTION Inflammation plays a significant role in the pathogenesis of pulmonary arterial hypertension (PAH). Soluble receptor activator of nuclear factor-κB ligand (sRANKL) and osteoprotegerin (OPG) are tumor necrosis factor α family members of immunomodulatory activity.

OBJECTIVES The aim of the study was to evaluate sRANKL and OPG concentrations in patients with PAH as potential factors contributing to the development of the disease.

PATIENTS AND METHODS We studied 26 patients with PAH, 31 volunteers, and 24 stable patients with chronic systolic left ventricular heart failure (LVHF) without pulmonary hypertension. The PAH group was followed up for 6 months for clinical deterioration or death.

RESULTS sRANKL levels were higher in the PAH group compared with controls and the LVHF group (5.6 [interquartile range, 3.9–7.9) vs. 2.0 [0.9–4.4] pmol/l; $P = 0.0001$, and 2.4 [1.3–4.2] pmol/l; $P = 0.001$, respectively). OPG levels were higher in PAH patients compared with controls (4.07 ±1.9 vs. 3.27 ±0.95 pmol/l; $P = 0.048$). We found significant correlations between sRANKL levels and parameters of ventilatory efficiency during exercise in the PAH group. OPG levels correlated with brain natriuretic peptide levels and with invasive hemodynamic parameters. Patients with clinical deterioration during 6-month follow-up (n = 9) showed higher baseline OPG levels compared with stable patients (n = 17, 5.09 ±2.6 vs. 3.52 ±1.19 pmol/l; $P = 0.043$). In the univariate analysis, the elevated OPG concentration at baseline was associated with an increased risk and earlier occurrence of clinical deterioration (hazard ratio, 1.43; 95% confidence interval, 1.06–1.9; $P = 0.017$).

CONCLUSIONS Concentrations of sRANKL and OPG are elevated in patients with PAH and are associated with indicators of disease severity and prognosis. sRANKL is a better discriminant between PAH and LVHF than OPG. The baseline OPG concentration is significantly associated with adverse outcomes in patients with PAH.

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INTRODUCTION Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by pulmonary vascular remodeling, leading to increased pulmonary arterial pressure and, subsequently, to right ventricular (RV) failure and death.[1] Chronic inflammation has been implicated in the development and progression of the disease.

Numerous studies have documented that patients with PAH have altered plasma levels or pulmonary expression of some cytokines and chemokines, eg, interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNF-α) and TNF-α superfamily members, and osteopontin as well as activation and infiltration into the vessel wall of inflammatory cells such as macrophages, T cells, B cells,

TABLE 1 Characteristics of the study groups

Parameter		PAH group (n = 26)	LVHF group (n = 24)	Control group (n = 31)
age, y		51.1 ±17.8	59.6 ±11.3	49.9 ±13.5
women, n (%)		18 (69.2)	3 (12.5)[a]	58 (18)
WHO/NYHA class, n (%)	II	6 (23.1)	4 (16.7)	
	III	14 (53.8)	18 (75)	
	IV	6 (23.1)	2 (8.3)	
BMI, kg/m^2		23.6 ±3.7	29.4 ±4[a]	25.8 ±3.5
systolic blood pressure, mmHg		120 ±16	121 ±12	126 ±15

Data are presented as mean ± standard deviation or number (percentage).

a $P < 0.05$ vs. the PAH group

Abbreviations: BMI – body mass index, LVHF – left ventricular heart failure, NYHA – New York Heart Association, PAH – pulmonary arterial hypertension, WHO – World Health Organization

and dendritic cells.[2-5] Recently, we have reported decreased serum levels of sTWEAK together with elevated concentrations of its scavenger receptor, sCD163, in patients with PAH, suggesting the role of these molecules in the pathobiology of the disease as components of the complex inflammatory process.[6]

The receptor activator of nuclear factor-κB ligand (RANKL) and its soluble decoy receptor—osteoprotegerin (OPG)—are TNF-α receptor family members involved in regulating bone turnover. OPG competitively inhibits an interaction between RANKL and its receptor resulting in inhibition of bone resorption.[7] Recent studies have reported elevated OPG levels in patients with high risk of atherosclerotic cardiovascular complications as well as in the PAH population and emphasized the prognostic significance of OPG.[8-10]

RANKL is expressed mainly by osteoblasts, but also by activated T helper cells and is thought to be involved in dendritic cell maturation and regulation of T cell-dependent immune response.[11] RANKL is a surface-bound molecule, but the soluble form (sRANKL) can be measured in serum. RANKL expression is regulated by various cytokines including IL-1, IL-6, IL-11, and TNF-α.[12] From the PAH perspective, there is a particularly interesting association between bone morphogenic proteins (BMPs) and RANKL. Impaired BMP signal transduction, particularly via the BMP-2 receptor, has been shown to predispose to PAH.[1] One of the described actions of the BMP-2 receptor is the induction of expression of RANKL, which might be important for PAH pathobiology; however, its importance has not been studied so far.[13]

Although growing evidence demonstrates the role of inflammatory processes in PAH pathogenesis, the knowledge regarding this issue is still limited. We hypothesized that OPG and RANKL play a role in the pathogenesis of PAH and their interaction may be important in the evaluation of patients with PAH and affect their prognosis. The aim of the present study was to evaluate sRANKL and OPG concentrations in patients with PAH as potential factors contributing to the development and progression of the disease.

PATIENTS AND METHODS

We investigated 26 subjects with PAH in functional classes II–IV according to the World Health Organization (WHO). These patients represented the whole population of patients who were diagnosed with PAH in north-eastern Poland and referred to the University Hospital of Bialystok (Białystok, Poland) between 2010 and 2013. The diagnosis of PAH was confirmed by right-sided heart catheterization on the basis of mean pulmonary artery pressure (mPAP) exceeding 25 mmHg and pulmonary capillary wedge pressure (PCWP) of less than 15 mmHg.

The reference cohort included 24 stable patients with chronic systolic left ventricular heart failure (LVHF) with no elevation in estimated mPAP and preserved systolic RV function, assessed by transthoracic echocardiography. The underlying cause of LVHF was classified as ischemic (n = 11) or idiopathic dilated cardiomyopathy (n = 13). The control group consisted of 31 volunteers (from outpatient clinics and general practice between 2010 and 2013) without pulmonary hypertension assessed by transthoracic echocardiography and without LVHF, matched for age, sex, body weight, and comorbidities with PAH subjects. Patients with acute infection, malignancy, and chronic obstructive pulmonary disease were not included in the study.

Study protocol

All patients underwent the same diagnostic assessment: initial determination of the WHO and New York Heart Association (NYHA) functional class, physical examination, transthoracic echocardiography, cardiopulmonary exercise test (CPET), 6-minute walk test (6MWD), and fasting venous blood tests. Patients with PAH were on modern specific therapy for PAH during 6-month follow up. Clinical deterioration (change in the WHO class; the need for escalation of therapy) and death were prespecified endpoints for clinical analysis in this study. The study was approved by the local research ethics committee and conducted according to the Declaration of Helsinki, and all subjects gave written informed consent to participate.

An echocardiography was performed to assess morphology and function of the right and left heart. Quantification of 2-dimensional and Doppler echocardiography data, including the RV basal diameter, right atrial area, left heart dimensions, degree of tricuspid regurgitation, and estimation of systolic pulmonary artery pressure were performed in a standard manner. RV systolic function was assessed by measuring the tricuspid annular plane systolic excursion (TAPSE) and percent fractional area change. Left ventricular systolic function was quantified by measuring left ventricular ejection fraction (LVEF) using the Simpson's method. Only patients with

TABLE 2 Hemodynamic data in patients with pulmonary arterial hypertension

systolic pulmonary artery pressure, mmHg	92.6 ±30.7
mean pulmonary artery pressure, mmHg	59.2 ±23
pulmonary capillary wedge pressure, mmHg	10.1 ±2.9
right atrial pressure, mmHg	9.1 ±3.8
cardiac output, l/min	4.1 ±1.1
cardiac index, l/min/m^2	2.5 ±0.8
pulmonary vascular resistance, Wood units	12.3 ±6.9
arterial oxygen saturation, %	92.6 ±4.1
mixed venous oxygen saturation, %	70.7 ±9.2

Data are presented as mean ± standard deviation.

TABLE 3 Biochemical, echocardiographic, and functional data

Parameter	PAH group (n = 26)	LVHF group (n = 24)	Control group (n = 31)
biochemical data			
BNP, pg/ml	266.5 (94–498)	108 (62–323)	27 (15–47)[a,b]
eGFR, ml/min/1.73m^2	77 ±18	75 ±18	87 ±14[b]
uric acid, mg/dl	6.25 ±2.4	6.54 ±1.4	4.6 ±1.5[a,b]
total cholesterol, mg/dl	167 ±41	176 ±43	219 ±37[a,b]
LDL cholesterol, mg/dl	105 ±37	109 ±36	140 ±36[a,b]
triglycerides, mg/dl	100 ±36	144 ±71[a]	121 ±63
echocardiographic data			
RV basal diameter, cm	4.8 ±1.2	3.3 ±0.9[a]	3.3 ±0.5[a]
right atrial area, cm^2	27 ±12	14 ±5[a]	11 ±3[a]
FAC, %	20 ±9.4	41 ±8.3[a]	43 ±3[a]
TAPSE, mm	18 ±3.9	24 ±5.6[a]	26 ±4[a]
eSPAP, mmHg	71.5 ±24.2	23.2 ±6.2[a]	13.4 ±7.4[a,b]
LVEF, %	58 ±5.3	24 ±5.3[a]	63 ±2.5[b]
exercise capacity			
peak VO_2, ml/kg/min	15.9 ±6	17 ±4.8	28.3 ±6.5[a,b]
6MWD, m	367 ±124	392 ±86	510 ±53[a,b]
ventilatory response to exercise			
VE/VCO_2	49 ±13	32 ±4[a]	26 ±4[a,b]
VE/VCO_2 slope	50.8 ±14.9	32 ±4.9[a]	25.7 ±3.7[a,b]
peak $PetCO_2$, mmHg	22.1 ±7.4	33.3 ±4.1[a]	39.6 ±6.4[a,b]

Data are presented as mean ± standard deviation or median (interquartile range).

a $P < 0.05$ vs. the PAH group
b $P < 0.05$ vs. the LVHF group

Abbreviations: 6MWD – 6-minute walk test, BNP – B-type natriuretic peptide, eGFR – estimated glomerular filtration rate, eSPAP – estimated systolic pulmonary artery pressure, FAC – fractional area change, LDL – low-density lipoprotein, LVEF – left ventricular ejection fraction, peak VO_2 – peak oxygen consumption, $PetCO_2$ – end-tidal partial pressure of carbon dioxide, RV – right ventricular, TAPSE – tricuspid annular plane systolic excursion, VCO_2 – ventilation equivalent for carbon dioxide, VCO_2 slope – slope of expired ventilatory flow vs. carbon dioxide production, others – see TABLE 1

normal RV parameters were included in the LVHF and control groups.

Exercise capacity and gas exchange parameters were obtained from CPET. It was performed using the maximum symptom-limited treadmill exercise with ramp protocol, as described previously.[14] The parameters used for analysis included peak oxygen uptake (peak VO_2), ventilation equivalent for carbon dioxide (VE/VCO_2), peak end-tidal partial pressure of CO_2 ($PetCO_2$) and the slope of the VE/VCO_2 relationship from the initiation to peak exercise (VE/VCO_2 slope).

Biochemical analysis Serum aliquots of 1.5 ml, after sample collection, were stored at –80°C for future analysis. Concentrations of OPG and sRANKL were measured by an enzyme-linked immunosorbent assay with commercially available kits (Biomedica, Austria). All measurements were performed in duplicate. The lower limits of detection were 0.07 pmol/l for OPG and 0.02 pmol/l for sRANKL. Serum diluted 1:10 was used for the assessment of the sRANKL concentration. Since OPG has been described as a decoy receptor for sRANKL signaling, we also analyzed the sRANKL/OPG ratio as a potential marker of free sRANKL signaling.

Statistical analysis To determine differences between the groups, 1-way analysis of variance with the Fisher's post hoc analysis and Mann–Whitney or Kruskal–Wallis with the Dunn's post hoc analysis were used depending on the distribution of variables. The Pearson or Spearman correlation coefficient was used to examine correlations between variables, as appropriate. To measure the associations between clinical outcome and OPG, the logistic regression model was used. The Cox proportional hazards analysis was performed to evaluate associations between the baseline values of numerous parameters and clinical deterioration during 6 months of follow-up (univariate analysis). Receiver operating characteristic (ROC) curves were used to establish the optimal values for differentiation of the analyzed subgroups. A P value of less than 0.05 was considered statistically significant.

RESULTS

The PAH group comprised patients with idiopathic PAH (46.2%, n = 12) as well as with PAH associated with connective tissue diseases and congenital heart diseases (26.9%, n = 7, both groups). Of these patients, 61.5% (n = 16) were prevalent cases receiving targeted treatment (7 patients on prostanoids); the rest (38.5%, n = 10) were incident cases. They were predominantly women. Patient characteristics are summarized in TABLE 1; hemodynamic data in the PAH group, in TABLE 2; and biochemical, functional, and echocardiographic data, in TABLE 3.

There were no significant differences in median brain natriuretic peptide (BNP) concentrations, mean 6MWD, and peak VO_2 consumption between the PAH and LVHF groups. Most patients had WHO/NYHA class III functional limitations. In an echocardiographic examination, PAH patients were characterized by indices of impaired RV morphology and global function with higher systolic pulmonary artery pressure and LVEF (TABLE 3). They also manifested a significantly lower ventilatory efficiency during exercise, characterized by elevated VE/VCO_2 and VE/VCO_2 slope

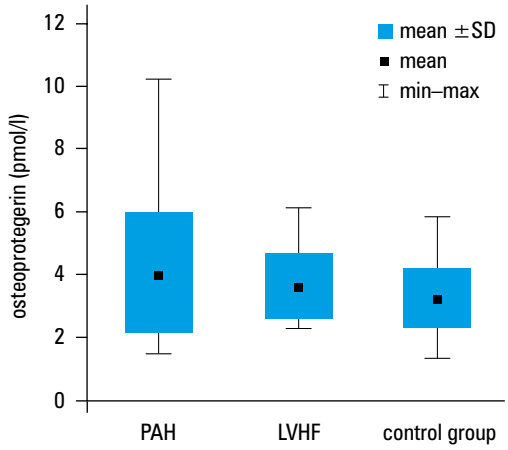

FIGURE 1 Osteoprotegerin concentrations in patients with pulmonary arterial hypertension (PAH), left ventricular heart failure (LVHF), and the control group

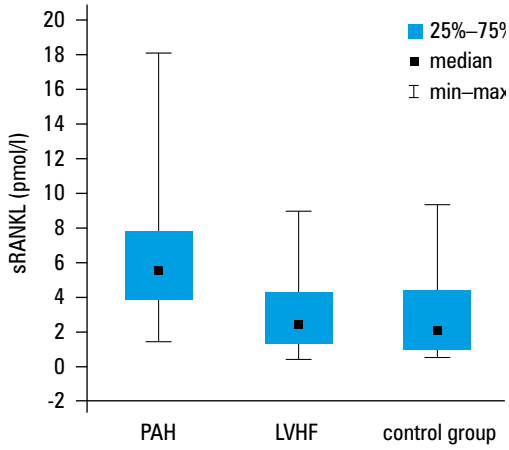

FIGURE 2 sRANKL concentrations in patients with pulmonary arterial hypertension (PAH), left ventricular heart failure (LVHF), and the control group

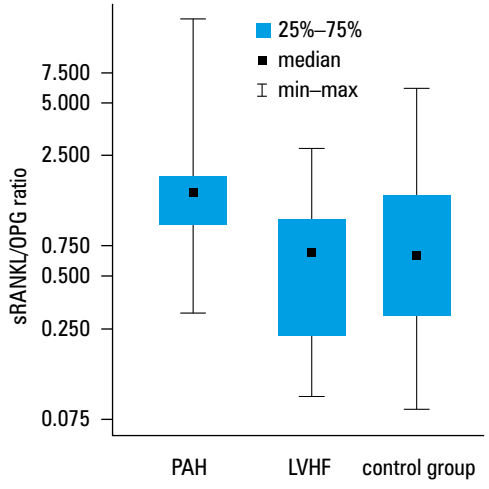

FIGURE 3 sRANKL/osteoprotegerin (OPG) ratio in patients with pulmonary arterial hypertension (PAH), left ventricular heart failure (LVHF), and the control group

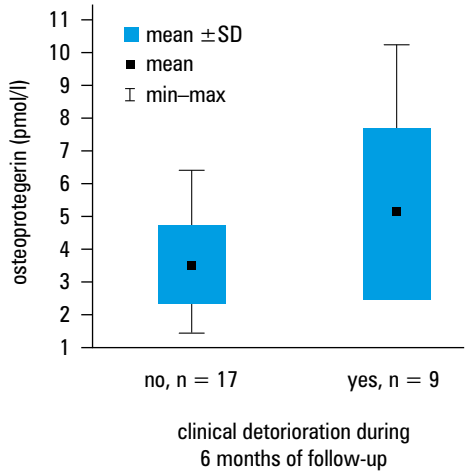

FIGURE 4 Osteoprotegerin concentrations in patients with pulmonary arterial hypertension in relation to clinical outcome during a 6-month follow-up

together with lower peak $PetCO_2$, when compared with the LVHF and control groups.

Patients with PAH had significantly higher mean serum OPG levels (4.07 ±1.9 pmol/l vs. 3.27 ±0.95 pmol/l; $P = 0.048$) compared with the control group (FIGURE 1). There was no significant difference in OPG concentrations between PAH and LVHF subjects (3.66 ±1.06 pmol/l; $P = 0.37$). Median sRANKL levels were significantly higher in the PAH group compared with controls and the LVHF group (5.2 [3.9–7.8] pmol/l vs. 2.0 [0.9–4.4] pmol/l, $P = 0.0001$, and 2.4 [1.3–4.2] pmol/l, $P = 0.001$, respectively, FIGURE 2). There was no difference in the concentrations of OPG and sRANKL between the LVHF and control cohorts. The sRANKL/OPG ratio was significantly higher in the PAH group compared with the other groups: median, 1.51 (0.3–15.34) in the PAH group vs. 0.65 (0.085–6.1) in the control group, $P = 0.004$, and vs. 0.68 (0.1–2.74) in the LVHF group, $P = 0.003$ (FIGURE 3). C-statistics obtained from the ROC curve analysis (in a pooled study population) showed that both the sRANKL concentration and sRANKL/OPG ratio have good diagnostic power for determining the presence of PAH (0.809, 95% confidence interval [CI], 0.714–0.904, with a cut-off value of 2.51 pmol/l for 92% sensitivity and 62% specificity, and 0.76, 95% CI, 0.653–0.866, with a cut-off value of 1 for 81% sensitivity and 71% specificity, respectively). OPG concentrations, however, did not differentiate patients with PAH and LVHF.

There were no significant differences in serum OPG and sRANKL concentrations and the sRANKL/OPG ratio between the groups of patients with different etiologies of PAH, between prevalent and incident cases (OPG, 4.14 ±2.27 vs. 3.93 ±1.2 pmol/l; sRANKL, 5.19 [interquartile range (IQR), 2.5–15.9] vs. 6.85 [IQR, 1.36–37.4] pmol/l; sRANKL/OPG 1.48 [IQR, 0.65–10.8] vs. 1.68 [IQR, 0.3–15.3]; $P > 0.05$) as well as in relation to the functional WHO class and sex.

We observed statistically significant correlations in the PAH group between sRANKL concentrations and right atrial pressure (RAP) ($r = 0.46$; $P < 0.05$), low-density lipoprotein cholesterol levels ($r = -0.41$; $P < 0.05$), total cholesterol levels ($r = -0.42$; $P < 0.05$), and parameters of ventilatory efficiency during exercise: VE/VCO_2 ($r = 0.45$; $P < 0.05$) VE/VCO_2 slope ($r = 0.49$; $P < 0.05$), and peak $PetCO_2$ ($r = -0.47$; $P < 0.05$). OPG levels positively correlated with BNP concentrations ($r = 0.7$; $P < 0.05$) and with hemodynamic parameters: cardiac output (CO; $r = -0.43$; $P < 0.05$), pulmonary vascular resistance (PVR) ($r = 0.43$; $P < 0.05$), mixed venous oxygen saturation ($r = -0.53$; $P < 0.05$), and RAP ($r = 0.49$; $P < 0.05$). Such correlations of sRANKL and OPG concentrations with biochemical and CPET parameters were not found in patients with LVHF and controls. No correlation was found between serum sRANKL and OPG concentrations in either group.

After 6 months of follow-up, clinical deterioration was observed in 9 patients (including

TABLE 4 Differences in baseline parameters between patients with pulmonary arterial hypertension with clinical deterioration and stable patients

	Patients with clinical deterioration, n = 9	Stable patients, n = 17	*P* value
OPG, pmol/l	5.09 ±2.6	3.52 ±1.19	0.043
sRANKL, pmol/l	6.3 (5.89–7.9)	4.5 (3.89–7.81)	NS
BNP, pg/ml	498 (377–1138)	121 (42–304)	0.009
6MWD, m	317 ±128	410 ±91	0.047
VE/VCO_2 slope	62 ±14.5	45.5 ±12.2	0.011
peak VO_2, ml/kg/min	12 ±3.7	17.7 ±6	0.03
SvO_2, %	65.2 ±10.1	73.8 ±7.3	0.02

Abbreviations: NS – nonsignificant, OPG – osteoprotegerin, sRANKL – soluble receptor activator of nuclear factor-κB ligand, SvO_2 – mixed venous oxygen saturation, others – see **TABLE 3**

2 deaths), while 17 patients remained stable. Patients with clinical deterioration showed significantly higher baseline OPG concentrations compared with stable subjects (**FIGURE 4**). There was no difference in baseline sRANKL levels between these groups. Differences in key baseline parameters between PAH patients with clinical deterioration and the stable group are presented in **TABLE 4**.

In a univariate logistic regression analysis, the concentration of OPG showed a trend towards prediction of clinical deterioration (P = 0.04 for the model and P = 0.089 for OPG). A more detailed univariate Cox analysis of proportional hazard showed that a higher OPG concentration at baseline was associated with increased risk and earlier occurrence of clinical deterioration during 6 months of follow-up (hazard ratio, 1.43; 95% CI, 1.06–1.9; P = 0.017). Other factors associated with adverse prognosis in a univariate Cox analysis were: SvO_2 (HR, 0.88; 95% CI, 0.81–0.96; P = 0.004), BNP (HR, 1.0; 95% CI, 1–1.002; P = 0.006), uric acid (HR, 1.52; 95% CI, 1.14–2.03; P = 0.004), peak VO_2 (HR, 0.86; 95% CI, 0.76–0.99; P = 0.036) and VE/VCO_2 slope (HR, 1.05; 95% CI, 1.01–1.1; P = 0.017). Neither the sRANKL or sRANKL/OPG ratio was associated with prognosis in any of the analysis.

DISCUSSION To the best of our knowledge, this is the first study to report elevated sRANKL levels in patients with PAH, compared with functionally similar patients with LVHF and controls. In addition, increased serum levels of sRANKL significantly correlated with the parameters of ventilatory efficiency during exercise and RAP, which are of prognostic significance and reflect disease severity.[1,15] Independently, sRANKL concentrations inversely correlated with the concentrations of total and LDL cholesterol, which may reflect the metabolic status of patients and peripheral organ dysfunction.

Since OPG is considered to be a decoy receptor for proinflammatory RANKL signaling, we decided to study both molecules simultaneously. In line with the previous studies,[8,9] we observed that patients with PAH have significantly higher serum OPG levels than controls but our study is the first to report a significant correlation between OPG and numerous hemodynamic markers of disease severity: CO, PVR, RAP, and SvO_2. Moreover, these data show that the baseline OPG concentration is a significant determinant of an adverse outcome in a short-term follow-up of patients with PAH. It appears that the sRANKL concentration is associated more closely with the presence of PAH, whereas OPG is less specific for this disease but carries more prognostic information, possibly owing to its closer association with RV function.

RANKL can modulate inflammatory and T-cell-dependent immune response, for example, by increasing endothelial-leukocyte cell interaction and endothelial permeability. Moreover, it was suggested to mediate inflammatory pathways during myocardial remodeling in heart failure.[16,17] With regard to PAH patients, describing the role of this molecule in the pathobiology of the disease is challenging owing to lack of relevant experimental and clinical studies. Thus, our data are innovative and might be epidemiologically important. Considering the available studies, as well as our data, we suggest that adaptive immune reactions occurring in the pulmonary vasculature in PAH are affected by sRANKL.

On the other hand, we showed significant correlations of sRANKL with the parameters of ventilatory efficiency during exercise. The studies in patients with chronic heart failure showed that reduced CO and associated chronic pathologies (neurohormonal, metabolic, and inflammatory imbalance) lead to skeletal and respiratory muscle damage and loss that augment exercise intolerance and ventilatory inefficiency.[18,19] Heart failure leads not only to myocyte damage but also to adipose tissue and bone loss.[19,20] RANKL activates osteoclasts, the cells involved in bone resorption. We showed inverse correlations of sRANKL with cholesterol levels, which may indirectly suggest or reflect metabolic disturbances (concerning muscle, adipose, and bone tissue loss) in the course of heart failure in patients with PAH. This could be supported by correlations of sRANKL and CPET parameters in the context of enhanced ventilatory response, intensified by muscle insufficiency. Interestingly, despite comparable clinical state in terms of the functional class in patients with LVHF, we observed neither elevation of sRANKL nor its correlation with ventilatory parameters and cholesterol. On the other hand, our LVHF patients had significantly higher mean body mass, suggesting less prevalent cachexia, and they more often received statins, which may inhibit RANKL expression.[21]

Previous studies suggested that OPG may act as an indirect indicator of pulmonary vasculature remodeling in PAH as it was found to be elevated in serum and pulmonary vascular lesions as well as to increase pulmonary artery smooth muscle cell proliferation and migration.[8,9] In our study, the OPG concentration correlated with hemodynamic parameters of right heart function and with BNP. This findings provide a rationale for a

hypothesis that OPG is secreted by myocytes of the right ventricle, but this concept requires further research. Experimental and clinical studies in patients with LVHF showed upregulation of OPG in the myocardium and immune system[22] and association of OPG levels with higher left ventricular mass, end-systolic volume, and lower LVEF.[23] Mizia-Stec et al.[24] have shown that elevated OPG levels characterized patients with severe aortic stenosis when compared with patients with a moderate disease. Moreover, the OPG concentration was higher in the presence of coronary artery disease in a cohort of subjects with mild aortic stenosis. Both these facts support the role of OPG as a potential marker of myocardial stress (ischemia, hypertrophy, or overload). The potential role of OPG in PAH pathogenesis was also supported by observations showing that OPG expression is regulated by BMPR2, 5-HT, and IL-1, all of which are associated with the pathogenesis of PAH.[8] It was suggested that OPG might be a mediator of inadequate or excessive complex inflammatory response in PAH. It is also considered to be a soluble inhibitor of RANKL signaling, which may promote inflammation and vascular remodeling. Nevertheless, the available studies do not provide a firm conclusion about the exact role of OPG in PAH. However, its contribution is highly likely.

We demonstrated that the OPG concentration is related to prognosis in a small group of patients with PAH. This supports previous reports and strengthens the above finding as we showed that it may be useful even in small populations. A significant finding in our study was not only the prognostic value of OPG, but also its relationship with numerous hemodynamic parameters of disease severity, which are also indicators of RV performance and overload. This underlines the role of OPG in PAH pathobiology. We did not show such an association with sRANKL, but we demonstrated its correlations with VE/VCO_2 and VE/VCO_2 slope, which have a prognostic value in patients with PAH.[15] When OPG is a prognostic parameter, sRANKL seems to act as a marker of the disease and may serve as a complementary molecule to OPG with respect to the pathogenic and prognostic role of the OPG-RANKL system in patients with PAH.

The study has several limitations including a relatively small sample size, single-center design, and short follow-up period. Moreover, the study group consisted of incident and prevalent cases and because of its small size, we were unable to assess the effect of disease-modifying therapy on sRANKL and OPG concentrations. Owing to a small cohort, we did not plan or attempt to perform a multivariate analysis of factors independently affecting clinical deterioration. Our ROC analysis was performed on a pooled study population consisting of 3 distinct groups and, therefore, it serves only as a preliminary analysis for hypothesis-generating purposes.

In conclusion, our study showed elevated levels of serum RANKL and OPG in the PAH population. Although we found both parameters to be elevated, only OPG provided short-term prognostic information. Considering that the OPG–RANKL system is altered in PAH patients and that sRANKL is more specifically associated with PAH and with other parameters reflecting disease progression, its potential prognostic role should not be discarded but rather investigated in a larger prospective trial in a nonbiased population.

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Contribution statement MJ and KK conceived the idea for the study. MJ and KK contributed to the design of the research. All authors were involved in data collection. MJ and KK analyzed the data. KK coordinated funding for the project. All authors edited and approved the final version of the manuscript.

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