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Enhanced IL-6 trans-signaling in pulmonary arterial hypertension and its potential role in disease-related systemic damage

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ABSTRACT

Background: The role of IL-6 in pulmonary arterial hypertension (PAH) has been reported but the prevalence of soluble receptors for IL-6: sIL-6R and sgp130 and its potential role in PAH have not been studied. Our aim was to examine the IL-6 together with the soluble receptors and to assess its relationship with clinical status of PAH patients as well as to assess its potential prognostic significance.
Methods: Serum concentrations of IL-6, sIL-6R and sgp130 were quantified by ELISA in 26 patients with PAH and 27 healthy controls and related to functional and biochemical parameters and clinical outcome in PAH group. The PAH patients were followed up for 1 year, noting the end point of clinical deterioration (WHO class change, the need for escalation of therapy) or death.
Results: The PAH group was characterized by higher median serum IL-6 [2.38 (IQR 1.56–3.75) vs 0.87 (0.63–1.3) pg/ml, $p = 0.000003$] and sIL-6R concentrations [69.7 (IQR 60.4–84.4 vs 45.7 (34.6–70.3) ng/ml, $p = 0.0036$] compared to control subjects. Both groups did not differ in sgp130 concentrations. There were significant correlations in PAH group between IL-6 levels and uric acid, parameters of ventilatory efficiency in cardiopulmonary exercise testing: VE/VO_2, VE/VCO_2, VE/VCO_2 slope and peak $PetCO_2$. sIL-6R levels inversely correlated with LDL cholesterol. After 1 year the clinical deterioration occurred in 11 patients, 15 remained stable. Patients in whom the clinical deterioration occurred showed significantly higher baseline concentrations of IL-6 [3.25 (IQR 2.46–5.4) pg/ml vs 1.68 (1.38–2.78) pg/ml, $p = 0.004$], but not sIL-6R. Median IL-6 $\geqslant$ 2.3 pg/ml (91% sensitivity, 73% specificity) identified subjects with worse clinical course. In the univariate analysis, higher IL-6 level at baseline was associated with increased risk and earlier occurrence of clinical deterioration (HR 1.42, 95%CI 1.08–1.85, $p = 0.015$).
Conclusions: IL-6 trans-signaling is enhanced in PAH. Elevated concentration of sIL-6R suggests its potential unfavorable role in systemic amplification of IL-6 signaling in PAH. Levels of IL-6 are associated with clinical indicators of disease severity as well as indirectly with systemic metabolic alterations. IL-6 shows prognostic value regarding predicting clinical deterioration.

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare progressive condition that results in right heart failure and premature death. Exercise intolerance is essential feature of the disease, mainly due to low cardiac output and respiratory dysfunction, but there is a growing evidence supporting the notion that exercise limitation in PAH might be attributed additionally to the muscle metabolism impairment [1].

The pathogenesis of PAH is complex and multifactorial, but over the past few years chronic inflammatory processes and dysregulation of multiple signaling pathways have been increasingly recognized as prominent pathogenic components of pulmonary vascular remodeling. Elevated circulating levels or pulmonary expression of multiple cytokines and chemokines, like IL-1β, IL-6, monocyte chemoattractant protein-1, osteopontin, osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (sRANKL), tumor necrosis factor alpha (TNF-α), sCD163 together with decreased serum levels of sTWEAK, as well as activation and infiltration of the vessel wall with macrophages, T and B lymphocytes and dendritic cells have been documented in patients with PAH [2–9].

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Moreover, our findings suggested indirect relationship of sRANKL concentration with musculoskeletal system function [9].

IL-6 is a multifunctional cytokine, produced by various cell types, such as macrophages, endothelial cells, vascular smooth muscle cells and fibroblasts. IL-6 exerts its biological activities by a heterodimeric receptor consisting of: specific membrane-bound or soluble IL-6 receptor (IL-6R, sIL-6R) and signal transducing receptor glycoprotein 130 (gp130). IL-6R is expressed only in few cell populations including leukocytes and hepatocytes, while gp130 is widely expressed in most cell types. When IL-6 binds to IL-6R, homodimerization of gp130 is induced, resulting in formation of functional receptor complex of IL-6/IL-6R/gp130, that initiates activation of intracellular signaling pathways. IL-6 binding to circulating sIL-6R allows for receptor complex formation with gp130 on cells that do not express IL-6R, what broadens the range of IL-6 affected cell types. This type of receptor signaling is called IL-6 trans-signaling [10]. In contrast to gp130, soluble form of gp130 (sgp130) is a natural inhibitor of IL-6 trans-signaling, as it prevents IL-6/sIL-6R binding to the membrane receptor. Given high affinity of IL-6 and sIL-6R with relatively high abundance of sIL-6R in plasma, IL-6 mediated effects largely depend on the concentration of the soluble receptors [10]. IL-6 trans-signaling pathway is considered to exert mainly pro-inflammatory actions [11].

The potential pathogenic role of IL-6 trans-signaling was reported in common cardiovascular diseases e.g. myocardial infarction and its complications [12], congestive heart failure, hypertrophic cardiomyopathy, but the prevalence of sIL-6R and sgp130 and its potential role in PAH have never been studied. As IL-6 mediated effect depends on its interplay with the receptors, we hypothesize that increased concentrations of soluble IL-6R in PAH could be important for the systemic amplification of IL-6 signaling in a variety of tissues. In this regard, skeletal muscle tissue might be of great importance. There is no unequivocal theory regarding pathophysiological background of PAH-related myopathy, but the chronic inflammatory condition is believed to have primary detrimental role [1]. It has been documented that IL-6 is a crucial regulator of muscle mass during cachexia [13].

The aim of our study was to assess the IL-6 together with its soluble receptors in patients with PAH in terms of IL-6 trans-signaling and examine the relationship between studied molecules and the hemodynamics, functional capacity and clinical status of these patients.

2. Material and methods

We conducted prospective single-center study that included 26 patients with PAH. The study group made a complete population of these patients in north-eastern Poland, referred to and treated in the University Hospital of Bialystok between 2010 and 2013. The diagnosis of PAH was based on established criteria [14] and confirmed by right-sided heart catheterization, using standard hemodynamic measurements: mean pulmonary artery pressure (mPAP) and pulmonary artery occlusion pressure (PAOP). The reference cohort consisted of 27 volunteers (from outpatient clinics and general practice between 2010 and 2013) matched for age, sex, body weight and comorbidities with PAH subjects. Patients with acute infection, malignancy, chronic obstructive pulmonary disease were not included in the study. None of participants took corticosteroids, non-steroid anti-inflammatory or immunosuppressive drugs.

2.1. Study protocol

All patients underwent the same diagnostic assessment: a medical interview with initial determination of the World Health Organization (WHO) functional class, physical examination, transthoracic echocardiography, cardiopulmonary exercise test (CPET), 6 minute walk test with distance assessment (6MWD) and fasting venous blood tests. The PAH patients were followed up for 1 year, noting the end point of clinical deterioration (WHO class change, the need for escalation of therapy) or death.

An echocardiography was performed to assess morphology and function of the right heart as well as to exclude any significant heart abnormalities in the control group. Quantification of two-dimensional and Doppler echocardiography data, including right ventricle and atrium dimensions, degree of tricuspid regurgitation and estimation of systolic pulmonary artery pressure (eSPAP) were performed in a standard manner [8]. Systolic function of the right ventricle was assessed by measuring the tricuspid annular plane systolic excursion (TAPSE) and percent fractional area change (FAC). Only patients with normal parameters of RV morphology and function as well as with preserved left ventricular ejection fraction were included in the control group.

CPET was performed using maximum, symptom limited treadmill exercise with ramp protocol in order to assess exercise capacity and gas exchange parameters. Of the numerous variables, peak oxygen uptake (peak VO_2), ventilation equivalents: VE/VO_2, VE/VCO_2, the slope of the VE/VCO_2 relationship from the initiation to peak exercise (VE/VCO_2 slope) and peak end-tidal partial pressure of CO_2 ($PetCO_2$) were used for analysis.

2.2. Blood sampling

Fasting peripheral venous blood samples were obtained from patients with PAH as well as controls. Serum aliquots of 1.5 ml were stored at −80 °C for future analysis. Concentrations of IL-6, sIL-6R and sgp130 were determined using commercially available ELISA kits (R&D Systems, USA) according to the manufacturer instructions. The mean minimum detectable value was 0.039 pg/ml for IL-6, 6.5 ng/ml for sIL-6R and 0.05 ng/ml for sgp130. All measurements were performed in duplicate. Serum diluted 1:100 was used for assessment of sIL-6R and sgp130 concentrations. Moreover we wanted to examine if there is any relationship of IL-6 and its receptors with other inflammatory molecules, previously assessed in our PAH population: circulating OPG and sRANKL. Concentrations of OPG and sRANKL were quantified by ELISA (Biomedica, Austria), as described previously [9]. All molecules were assessed from the blood samples collected at the same time point. Blood samples were also analyzed for B-type natriuretic peptide (BNP), C-reactive protein (CRP), uric acid, cholesterols, white blood cells, platelets count in the local laboratory of University Hospital of Bialystok.

The study was approved by the institutional medical ethics committee. All patients gave written informed consent for participating the study, including taking and storage of blood samples. The study complied with the declaration of Helsinki.

2.3. Statistical analysis

The distribution of all variables was verified with Kolmogorov–Smirnov test. Data are expressed as mean ± standard deviation (SD) or median values with interquartile range (IQR) as appropriate. Statistical analysis was performed using *t*-student or Mann–Whitney *U* test for continuous data depending on distribution and χ^2 test for categorical variables. Spearman's or Pearson's correlation coefficient was used to examine the relationship between 2 continuous variables. To measure relationship between clinical outcome and studied molecules the logistic regression model was used. Cox proportional hazards analysis was performed to evaluate association between baseline values of numerous parameters and clinical deterioration during 1 year of observation