Clinical Investigations

Respiration

Respiration
DOI: 10.1159/000488908

Received: November 14, 2017
Accepted after revision: March 27, 2018
Published online: June 15, 2018

Neopterin as a Biomarker in Patients with Pulmonary Arterial Hypertension and Chronic Thromboembolic Pulmonary Hypertension

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Keywords

Neopterin · Inflammation · Prognostic biomarker · Pulmonary arterial hypertension · Chronic thromboembolic pulmonary hypertension

Abstract

Background: Upregulation of the immune system is regarded to play an important role in the etiopathobiology of pulmonary arterial hypertension (PAH) and inoperable chronic thromboembolic pulmonary hypertension (CTEPH). To the best of our knowledge, neopterin (NP) has never been investigated in patients with PAH and CTEPH. ***Objectives:*** The aim of the study was to evaluate the concentration of NP in blood in order to examine its impact on outcome and relationship with disease severity in that population. ***Methods:*** Serum concentration of NP was analysed prospectively in 50 patients (36 with PAH and 14 with CTEPH vs. 31 healthy controls) and assessed in relation to clinical parameters and outcome. ***Results:*** NP concentration in the PAH and CTEPH groups combined was significantly higher than in the control group (8.68, 6.39–15.03 vs. 5.14, 4.16–5.98 nmol/L, $p < 0.0000001$). During 9 months of follow-up, clinical deterioration occurred in 18 patients (including 8 deaths), and NP concentration in this group was higher when compared to stable patients (15.6, 8.52–25.13 vs. 7.87, 6.18–9.89, $p = 0.002$). The cutoff value of NP derived from ROC curve analysis was 15.3 nmol/L ($p = 0.002$, AUC 0.77, $p = 0.0004$, HR = 4.35, 95% CI 1.43–13.18, log-rank test). On Cox regression analysis, NP predicted clinical deterioration ($p = 0.009$, 95% CI 1.01–1.06). NP correlated positively with NT-proBNP ($p < 0.001$), red blood cell distribution width ($p < 0.001$), and right atrium area ($p = 0.002$) and inversely with 6-min walking test ($p = 0.002$) and peak oxygen consumption ($p = 0.001$). ***Conclusions:*** NP concentration is increased in patients with PAH and inoperable CTEPH. Elevated NP concentration is associated with adverse clinical outcomes and correlates with clinical parameters.

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Introduction

Chronic upregulated inflammatory response has been widely acknowledged as a crucial pathogenic element of pulmonary arterial hypertension (PAH) and, to a lesser extent, inoperable chronic thromboembolic pulmonary hypertension (CTEPH). Moreover, in the literature, there is a large amount of evidence indicating that specific abnormalities in the immune system play an important role in disease progression by stimulating inflammatory cell recruitment. These activated cells that include macrophages, T and B lymphocytes, dendritic cells, and mast cells infiltrate to the perivascular area and release numerous cytokines and chemokines, which promotes proliferation and migration of pulmonary vascular cells as well as autoantibody formation, which in turn results in vasoconstriction and matrix deposition [1].

Elevated levels of various proinflammatory cytokines, such as Il-1, Il-6, TNF-α, Il-13 [2], Il-2, Il-4, Il-8, Il-10, Il-12 [3], and transforming growth factor beta (TGF-β) [4], were observed in PAH. In addition, Le Hiress et al. [5] documented that macrophage migration inhibitor factor (MIF) and its receptor CD74 are increased in patients with idiopathic PAH (IPAH), contributing to the proinflammatory phenotype of pulmonary endothelial cells. Furthermore, elevated mRNA expression of the chemokine RANTES, a chemoattractant for monocytes and T cells [6], as well as CC-chemokine ligand 2 (CCL2), which can stimulate macrophage migration and smooth muscle cell proliferation, was demonstrated in lungs of patients with PAH [7]. Ulrich et al. [8] showed an increased level of circulatory CD4+CD25^{high} (so called regulatory T cells involved in tolerance and anergy) and a decreased level of cytotoxic CD8 T cells in patients with IPAH compared to controls. This specific imbalance in the subpopulation of T cells may contribute to the vascular remodelling and disease progression. Additionally, serum concentrations of numerous inflammatory cytokines were documented as elevated in operable and inoperable CTPEH: C-reactive protein, Il-10, monocyte chemotactic protein-1, macrophage inflammatory protein-1, metalloproteinase (MMP)-9 [9], Il-1, Il-4,Il-8, Il-10 [10], TNF-α [11], as well as CCl-2 and chemokine CXC ligand 13 (CXCL13): B-lymphocyte-chemoattractant (BLC) [12].

Neopterin (NP) is an early biomarker of cellular immune response and belongs to the class of pteridines. It is released by activated macrophages and dendritic cells after stimulation with interferon-γ (INF-γ) via guanosine triphosphate (GTP) cyclohydrolase I [13]. Elevated concentration of NP has been documented in various clinical states, whose common feature is dysregulation of the immune system and chronic inflammation. Several studies showed increased concentration of NP in cardiovascular diseases [14, 15], such as coronary artery disease [16], heart failure with reduced [17] and preserved ejection fraction [18], open heart operations [19], various infection diseases [20], malignancy [21] as well as in patients after transplantation as an early biomarker of allograft rejection [22]. In addition, in the above-mentioned studies, NP was documented as a biomarker predicting death and/or serious adverse events. However, serum NP concentration has not been investigated to date in PAH and inoperable CTEPH. The aim of this study was to determine NP serum concentration and to examine its impact on outcome and relationship with disease severity in patients with PAH and inoperable CTEPH.

Materials and Methods

Fifty patients (PAH, $n = 36$; CTEPH, $n = 14$) and 31 healthy controls were enrolled in this prospective single-center study. The PAH group included patients with IPAH (45%, $n = 16$) and PAH associated with congenital heart disease (33%, $n = 12$), connective tissue disease (19%, $n = 7$), and portopulmonary arterial hypertension (3%, $n = 1$). Most patients had WHO Functional Class (FC) II and were female. Detailed demographics and baseline characteristics are shown in Table 1.

The study group included the whole population of patients in western Poland treated with PAH- and CTEPH-targeted drugs at the Poznan University of Medical Sciences in 2016–2017. The diagnosis of PAH and CTEPH was based on standard criteria [23] and confirmed by right heart catheterization. Hemodynamic data and targeted treatment are presented in Table 2. Operability of vascular lesions in CTEPH was assessed by a multi-expert team including a thoracic surgeon experienced in pulmonary artery thromboendarterectomy (PEA) and an invasive cardiologist trained in balloon pulmonary angioplasty. When the vascular lesions in CTPEH were classified as inoperable, the balloon pulmonary angioplasty procedure was performed and simultaneously treatment with riociguat was introduced. Moreover, all patients underwent diagnostic assessment on a regular basis, every 3–6 months: blood sample collection for routine analysis, medical interview with determination of the WHO FC, physical examination, transthoracic echocardiography, 6-min walking test (6MWT), and cardiopulmonary exercise test (CPET). The end point of clinical deterioration comprised unscheduled hospitalization due to disease progression, WHO FC change, the need for escalation of therapy, or death.

Blood Sampling and Assay

Peripheral venous blood samples were obtained from patients with PAH and CTEPH as well as healthy volunteers as controls, then centrifuged at 10,000 g for 10 min and stored at −80 °C for future analysis. Serum NP was assessed by enzyme immunoassay Neopterin ELISA DRG International, Inc., USA.

Table 1. Patient demographics

Characteristics	PAH (n = 36)	CTEPH (n = 14)	Control (n = 31)	p value[1]
Gender, n (%)				0.017
Female	28 (78)	9 (64)	14 (45)	
Male	8 (22)	5 (36)	17 (55)	
Age, years	55 (18–78)	66.5 (27–79)	44 (25–60)	<0.001
PAH etiology, n (%)				
IPAH	16 (45)			
PAH CHD	12 (33)			
PAH CTD	7 (19)			
PAH PoP	1 (3)			
WHO FC, n (%)				
I	3 (8)	0 (0)		
II	18 (50)	7 (50)		
III	13 (36)	6 (43)		
IV	2 (6)	1 (7)		
PeakVO_2, mL/kg/min	14.7±4.6	13.9±3.5		
6MWT, m	404±159	318±164		
Right atrium area, cm^2	29±13	23±8		
Fluid in pericardium, n (%)	3 (8)	0 (0)		
NT-proBNP, pg/mL	604 (156–2,695)	449 (142–1,608)		
RDW, %	14.9±1.9	15.2±2.0		

PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; IPAH, idiopathic pulmonary arterial hypertension; PAH CHD, pulmonary arterial hypertension associated with congestive heart defect; PAH CTD, pulmonary arterial hypertension associated with connective tissue disease; PAH PoP, portopulmonary arterial hypertension; WHO FC, World Health Organization Functional Class; peakVO_2, peak oxygen consumption; 6MWT, 6-min walking test; NT-proBNP, N-terminal B-type natriuretic peptide; RDW, red blood cells distribution width.

[1] p value is given for patients with PAH and CTEPH combined versus controls.

Table 2. Hemodynamic data and targeted treatment

	PAH (n = 36)	CTEPH (n = 14)
Hemodynamic parameters (mean ± SD)		
Pulmonary artery pressure, mm Hg	51±13	46±9
Pulmonary vascular resistance, Wood units	7.2±3.4	5.6±2.6
Cardiac index, $L/min/m^2$	3.14±0.67	3.49±0.71
Right atrium pressure, mm Hg	7.5±4.9	9.0±5.7
Mixed venous oxygen saturation, %	68.8±6.5	69.1±7.3
PAH- and CTEPH-targeted treatment		
Monotherapy, n (%)		
Riociguat	0 (0)	14 (100)
Sildenafil	6 (16.5)	0 (0)
Bosentan	6 (16.5)	0 (0)
Combined therapy, n (%)		
Sildenafil + treprostinil	9 (25)	0 (0)
Sildenafil + macicentan	8 (22)	0 (0)
Sildenafil + iloprost	4 (11)	0 (0)
Sildenafil + bosentan	1 (3)	0 (0)
Sildenafil + epoprostenol	1 (3)	0 (0)
Sildenafil + iloprost+ bosentan	1 (3)	0 (0)
Baloon pulmonary angioplasty	0 (0)	14 (100)

PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension.

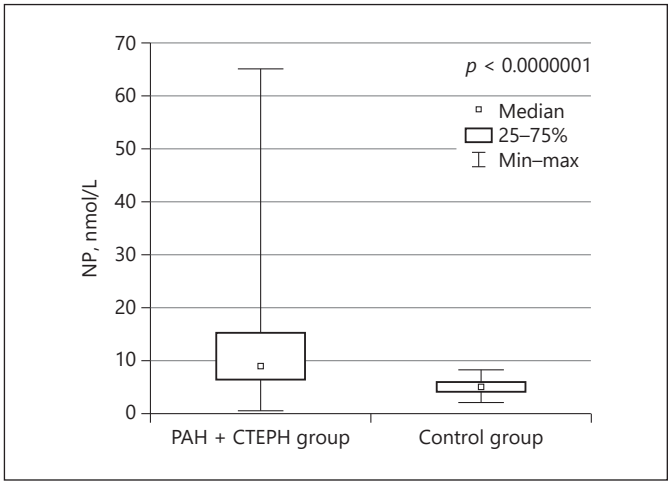

Fig. 1. The diagram presents a significantly higher concentration of neopterin (NP) in the PAH and CTEPH population compared to controls.

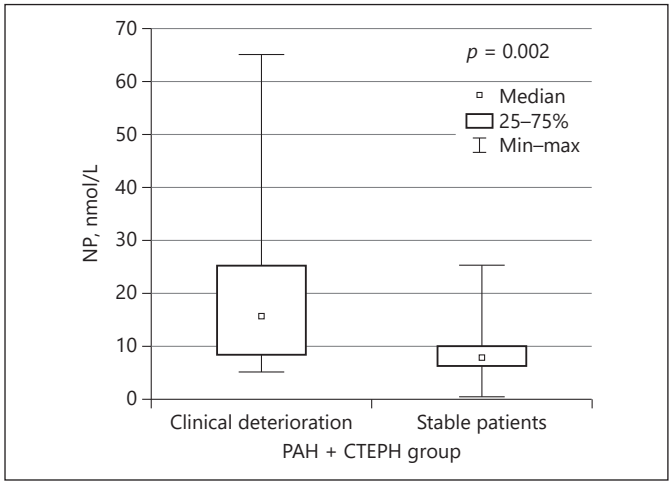

Fig. 2. The diagram presents a significantly higher concentration of neopterin (NP) in patients with clinical deterioration compared to stable patients.

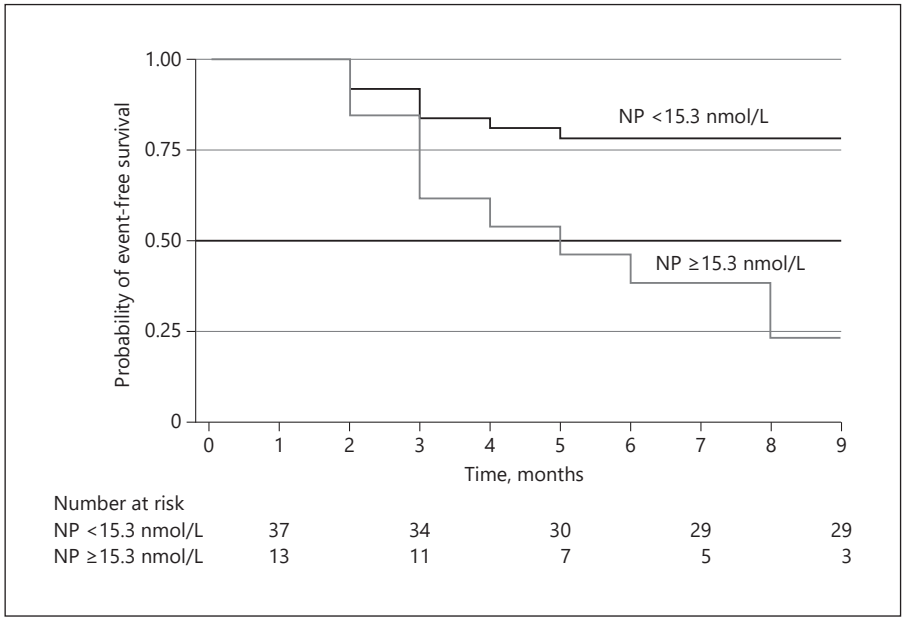

Fig. 3. Kaplan-Meier survival curves showing survival estimates in patients with pulmonary arterial hypertension or chronic thromboembolic pulmonary hypertension, graded by neopterin (NP) level below and above receiver operating characteristic-derived values during 9 months of follow-up.

Written informed consent was obtained from all patients and controls, and the Poznan University of Medical Sciences ethics committee approved this study (the number of ethic approval: 966/16).

Statistical Analysis

The distribution of all variables was verified with the Shapiro-Wilk test for normality. Data are shown as mean ± SD or median values with IQR as appropriate. Statistical analysis was performed using the *t* test or Mann-Whitney U test for continuous data depending on distribution and the χ^2 test for categorical variables. Associations between NP and clinical and hemodynamic variables were assessed with Spearman's correlation analysis. Receiver operating characteristics (ROC) curve was used to determine a cutoff point associated with a higher probability of clinical deterioration. A survival curve was plotted as estimated by the Kaplan-Meier method and compared with others using the log-rank test. We assessed the impact of the level of NP on clinical deterioration by creating a Cox proportional hazard model. p values <0.05 were considered statistically significant. Statistical analysis was performed using the PQStat version 1.6.2.

Table 3. Serum neopterin concentrations in subgroups

	Neopterin, nmol/L
PAH + CTEPH (n = 50)	8.68 (6.39–15.03)
PAH (n = 36)	9.04 (7.21–20.40)
CTEPH (n = 14)	7.5 (6.1–10.0)
IPAH (n = 16)	9.19 (7.67–16.73)
Control (n = 31)	5.14 (4.16–5.98)

PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; IPAH, idiopathic pulmonary arterial hypertension.

Results

NP concentration in the PAH and CTEPH groups combined was significantly higher than in the control group (8.68, 6.39–15.03 vs. 5.14, 4.16–5.98; Fig. 1). During 9 months of follow-up, clinical deterioration occurred in 18 patients (including 8 deaths), and NP concentration in this group was significantly higher when compared to stable patients (15.6, 8.52–25.13 vs. 7.87, 6.18–9.89, p = 0.002; Fig. 2). The cutoff value of NP derived from ROC curve analysis was 15.3 nmol/L (p = 0.002, AUC 0.77, 95% CI 0.63–0.91; log-rank: p = 0.0004, HR = 4.35, 95% CI 1.43–13.18) (Fig. 3). On Cox regression analysis, NP predicted clinical deterioration (p = 0.009, 95% CI 1.01–1.06). NP correlated (Fig. 4) positively with N-terminal pro-B-type natriuretic peptide (NT-proBNP, p < 0.001), red blood cell distribution width (RDW, p < 0.001), and right atrium area derived from echocardiography (p = 0.002) and inversely with 6MWT (p = 0.002) and peak oxygen consumption (peakVO_2, p = 0.001). WHO FC and hemodynamic parameters including mean pulmonary pressure, cardiac index, mean right atrium pressure, mixed venous saturation, and pulmonary vascular resistance showed no significant correlation with NP (p = 0.17, p = 0.9, p = 0.42, p = 0.57, p = 0.42, and p = 0.069, respectively). In the analysis of NP concentration in the PAH and CTEPH group separately versus the control group, we found a significant difference in both groups (9.04, 7.21–20.40 vs. 5.14, 4.16–5.98 nmol/L, p = 0.001, for the PAH group and 7.48, 6.10–10.02 vs. 5.14, 4.16–5.98 nmol/L, p < 0.001, for the CTEPH group). There was no significant difference in NP concentration in the CTEPH versus PAH group (p = 0.15; Fig. 5). In further subgroup analyses of NP concentration in the IPAH group compared to the control group, we documented a significant difference as well (9.19, 7.67–16.73 vs. 5.14, 4.16–5.98 nmol/L, p < 0.001) and NP concentration predicted clinical deterioration (log rank: p = 0.017, HR 5.41, 95% CI 1.10–26.60) in this group of patients. The summary of the results of serum NP concentrations in the individual groups of patients is presented in Table 3. In addition, we observed a positive correlation of NP concentration with age in patients with PAH (p = 0.019), while there was no significant correlation of NP with age in patients with CTEPH as well as in the control group (p = 0.11 and p = 0.85, respectively).

Discussion

NP increases the cytotoxic potential of activated macrophages and dendritic cells [24]. The key biochemical role of NP is most likely the interaction with reactive oxygen or nitrogen intermediates, thereby promoting oxidative stress [25, 26]. The interaction of NP with the intermediates and its ability to amplify the effects of various reactive oxygen species might be of importance for PAH and inoperable CTEPH progression. Moreover, NP production at the expense of tetrahydrobiopterin (BH4), a cofactor of nitric oxide synthase (NOS), leads to BH4 deficiency and finally to NOS decomposition and reactive oxygen (O_2^-) creation. Subsequently, O_2^- reacts with nitric oxide (NO), which results in peroxynitrite ($ONOO^-$) formation, and then $ONOO^-$ inactivates BH4 by oxidation [24, 27] (Fig. 6). As a result of this process, the production of NO is limited in human macrophages and may be compensated by endothelial NO formation. It is well established that dysregulation of the NO pathway, the crucial vasodilator of endothelial cells is regarded to be a key element of etiobiology of PAH onset and development. Furthermore, there is a large body of evidence that oxidative stress contributes to PAH pathogenesis. Elevated levels of urinary F2-isoprostanes, a biomarker of lipid peroxidation, was documented in patients with PAH and correlated inversely with vasoreactivity [28]; F2-isoprostanes decreased in urine after epoprostenol treatment and correlated with hemodynamic and clinical improvement [29]. Oxidized guanosine was also elevated in lungs of patients with PAH [30]. In animal models, in mutant bone morphogenetic protein receptor 2 mice, hyperoxia contributed to disease exacerbation [31]. Another mechanism in which NP may play a role in PAH progression is its ability to induce nuclear factor-κB (NF-κB) translocation in human en-

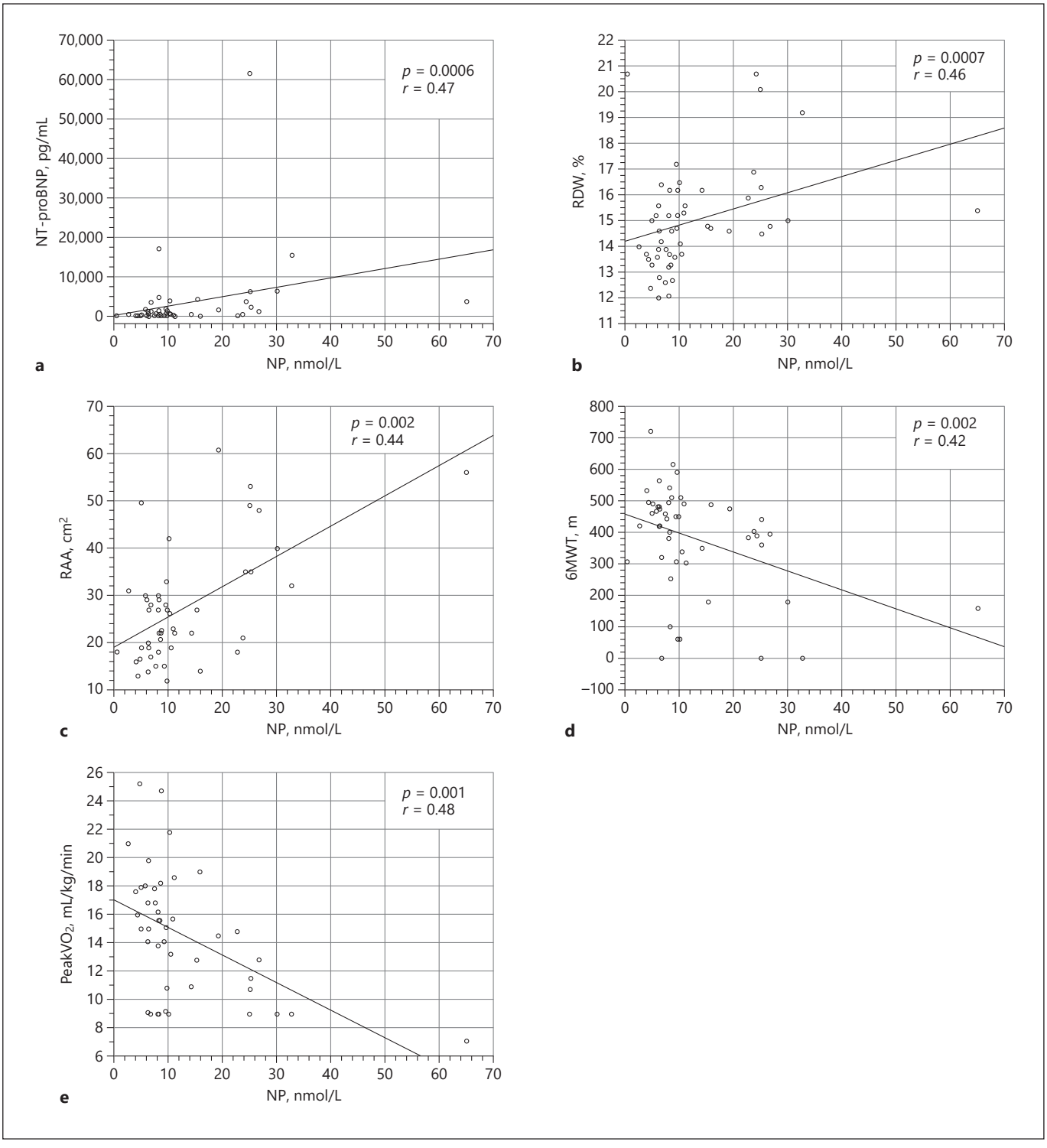

Fig. 4. a–e Correlations between neopterin (NP) and clinical parameters. **a** NP and N-terminal pro-B-type natriuretic peptide (NT-proBNP). **b** NP and red blood cell distribution width (RDW). **c** NP and right atrium area (RAA). **d** NP and 6-min walking test (6MWT). **e** NP and peak oxygen consumption (peakVO_2).

dothelial cells [32]. In a recent study in animal models of PAH, miR-130a (microRNA), modulated by NF-κB, was found overexpressed in lung microvascular endothelial cells [33]. Finally, it is well known that patients with PAH and CTEPH, especially in advanced stages of the disease, similarly to patients with malignancies, suffer from cachexia, weight loss, and muscular dystrophy, which is associated with an elevated inflammatory response mediated by various cytokines and increased catabolism of tryptophan. Hence, an elevated concentration of NP and a decreased tryptophan concentration was demonstrated in patients with weight loss and hematological tumors [34]. These findings could be explained by INF-γ, which stimulates both NP production (Fig. 5) and tryptophan degradation by stimulating the enzyme indoleamine-2,3-dioxygenase (IDO).

In our study, for the first time, we have shown that NP serum concentration is elevated in PAH and inoperable CTEPH, and the cutoff value for NP of 15.3 nmol/L differentiates patients with a high probability of clinical deterioration including death. In addition, the risk of clinical deterioration in the subgroup with a NP concentration ≥15.3 nmol/L was more than 4-fold higher than in the subgroup with a lower concentration of NP. In previous studies, a cutoff value of 14.5 nmol/L in surgical patients predicted postoperative complications after operations with cardiopulmonary bypass [35], while in patients after an acute coronary syndrome event, an NP level ≥12.11 nmol/L was associated with an increased risk of death and/or acute coronary events after adjustment for clinical variables [16]. Nevertheless, we measured serum NP concentration which reflects an upregulated inflammatory response in the whole circulatory system; therefore, we can only hypothesize that production of NP locally in lung tissue, where specific inflammatory infiltrates in the perivascular space are found, is

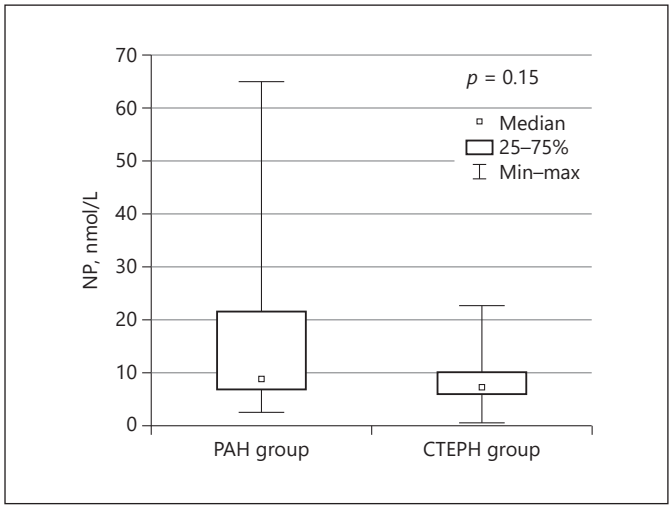

Fig. 5. The diagram presents no significant difference in neopterin (NP) concentration in the inoperable CTEPH versus PAH group ($p = 0.15$).

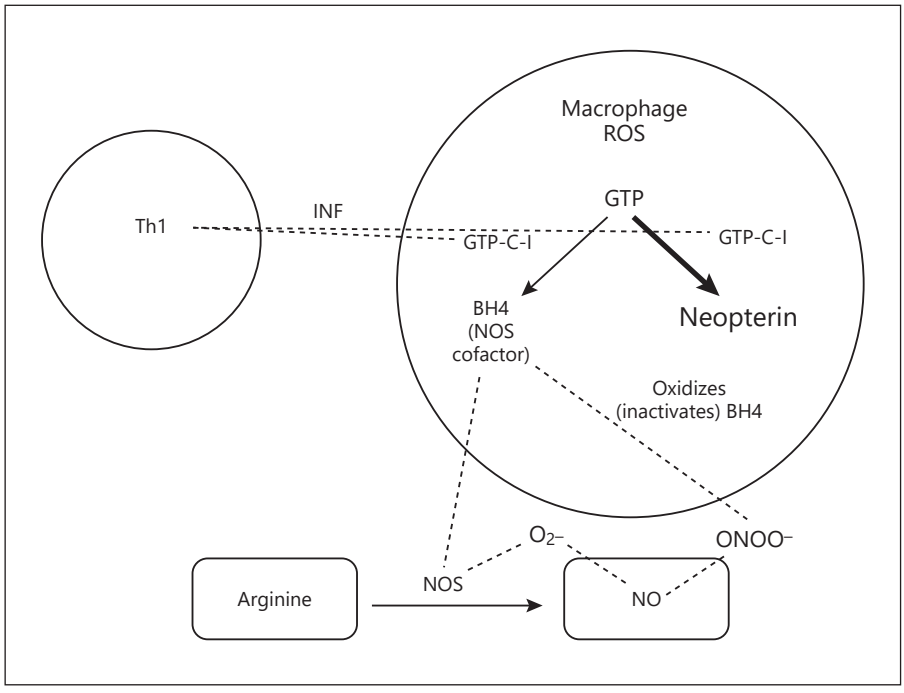

Fig. 6. The scheme presents cellular immune response during inflammation and the role of neopterin (NP) in oxidative stress enhancement. Interferon-γ (INF), produced by lymphocytes Th1, stimulates guanosine triphosphate (GTP)-cyclohydrolase I (GTP-C-I) in activated macrophages. NP production at the expense of tetrahydrobiopterin (BH4), a cofactor of NOS, leads to BH4 deficiency and finally to NOS decomposition and O_2^- creation. Subsequently, O_2^- reacts with nitric oxide (NO), which results in peroxynitrite ($ONOO^-$) formation, and then $ONOO^-$ inactivates BH4 by oxidation. ROS, reactive oxygen species.

significantly elevated as well. According to the guidelines of the European Society of Cardiology, each patient with PAH or inoperable CTEPH should be assessed every 3–6 months to check whether the patient fulfils the criteria of disease stability [23]. Thus, we investigated NP serum concentration with regard to these validated parameters (clinical, functional, hemodynamic as well as biomarker: NT-proBNP) and we report a positive correlation of NP with NT-proBNP, and right atrium area and an inverse correlation with 6MWT and peak-VO_2 assessed in CPET. Nowadays, NT-proBNP is the only biomarker which allows to estimate the risk of 1-year mortality in patients with PAH and CTEPH. However, it is the specific parameter for hemodynamic function and it represents the severity of heart failure. Hence, serum NP, the inflammatory biomarker, may have additional value in prognostication regarding the inflammatory background of the disease. Moreover, we demonstrated a positive correlation of NP concentration with RDW, a simple parameter measured in standard blood analyses, which recently has been described as a predictive biomarker in patients with PAH and inoperable CTEPH [36]. However, the positive correlation of NP with NT-proBNP and RDW could be a result of a couple of outliers because of a wide range of values of these parameters (19.2–61,647.0 pg/mL for NT-proBNP and 12.0–20.7% for RDW). In addition, WHO FC and hemodynamic parameters including mean pulmonary pressure, cardiac index, mean right atrium pressure, mixed venous saturation, and pulmonary vascular resistance showed no significant correlation with NP. However, the treatment of the entire study population with targeted drugs significantly affected hemodynamic results and may explain the lack of correlation of NP with hemodynamic parameters. Additionally, our findings support the assumption that the right ventricle function and its adaptation to increased pulmonary pressure reflected by functional tests (such as 6MWT and CPET) and biomarkers (such as NT-proBNP) are crucial variables and may have greater impact on the disease prognosis than hemodynamic results. Nevertheless, the regular comprehensive assessment of the patient should contain both noninvasive and invasive parameters.

Furthermore, regarding the heterogeneity of the study group, we examined NP concentration in individual groups: CTEPH group compared to control group, PAH group compared to control group, and IPAH group compared to control group, which showed significant differences in all analyses. We also evaluated NP concentration in the CTEPH group compared to the PAH group, which revealed no significance difference, confirming the hypothesis of an upregulated inflammatory response in both diseases. Finally, we found a positive correlation of NP concentration with age in the PAH group, which indicates that the concentration of NP increases with age. Nevertheless, in the CTEPH group and control group, there was no significant correlation of NP concentration with age.

Our results confirm the crucial involvement of sustained immune activation, especially of macrophages, in PAH and inoperable CTEPH progression. Thus, NP, a marker of cellular inflammatory mechanisms that contribute to vascular remodelling, may serve as a potential predictive biomarker of disease severity as well as clinical worsening.

Limitations

Study limitations include a relatively small cohort size as well as the fact that subjects in the control group were significantly younger and were predominantly males, which can slightly affect the NP results in both groups. There is a need to conduct studies with a larger number of patients to validate serum NP as an independent prognostic biomarker.

Conclusions

Serum NP concentration is elevated in patients with PAH and inoperable CTEPH. In addition, elevated NP concentration is associated with adverse clinical outcomes and correlates with clinical parameters. The results of our study and the role of NP in oxidative stress enhancement indicate that NP may be a candidate biomarker of PAH and CTEPH deterioration.

Disclosure Statement

The authors declare that there is no conflict of interest.

Funding Sources

This research received no grant from any funding agency in the public, commercial, or not-for-profit sectors

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