Platelet sTWEAK and plasma IL-6 are associated with 18F-fluorodeoxyglucose uptake in right ventricles of patients with pulmonary arterial hypertension: A pilot study

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Abstract

Background. Cytokines soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) and interleukin 6 (IL-6) are involved in immune response, proliferation, apoptosis, and cardiovascular pathologies. We have previously confirmed that changes of their platelet or plasma contents are associated with pulmonary arterial hypertension (PAH). The positron emission tomography/magnetic resonance imaging (PET/MRI) hybrid imaging provides detailed insight into right ventricle (RV) hemodynamic and metabolic function.

Objectives. To evaluate the relationship between RV parameters obtained using PET/MRI and concentrations of plasma and platelet sTWEAK and IL-6 in stable PAH patients.

Materials and methods. Eighteen stable PAH patients (48.44 ±16.7 years) had simultaneous PET/MRI scans with 18F-fluorodeoxyglucose (^{18}F-FDG) performed. Its uptake was presented as a standardized uptake value (SUV) for RV and left ventricle (LV). Cytokines concentrations were measured in platelet-poor plasma and platelet lysate. Follow-up time of this study was 58 months; the combined endpoint (CEP) was defined as death or clinical deterioration.

Results. We observed significant correlations between platelet sTWEAK levels, plasma IL-6 and PET parameter $SUV_{RV/LV}$ ($r = -0.57$, $p = 0.011$; $r = 0.50$, $p = 0.032$, respectively). In logistic regression, platelet sTWEAK and IL-6 were both prognostic factors for unfavorable ratio of $SUV_{RV/LV}$ higher than 1 (hazard ratio (HR) = 0.44, 95% confidence interval (95% CI): [0.23; 0.84], $p = 0.017$; and HR = 3.62, 95% CI: [1.21; 10.17], $p = 0.011$, respectively). Furthermore, their concentrations were related with prognostically important higher late gadolinium enhancement mass index (LGEMI) and RV global longitudinal strain/systolic pulmonary artery pressure (RV GLS/sPAP) values. Patients who had CEP in follow-up ($n = 13$) had significantly lower platelet sTWEAK content and higher plasma IL-6 at baseline than stable patients. Lower platelet sTWEAK was related to a worse prognosis in log-rank test ($p = 0.006$). Platelet sTWEAK and plasma IL-6 together with RV GLS/sPAP, RV ejection fraction (RVEF), mean pulmonary arterial pressure (mPAP), and $SUV_{RV/LV}$ were significantly associated with time to CEP in univariate Cox analysis.

Conclusions. The sTWEAK and IL-6 concentrations in PAH patients are linked with metabolic and functional changes of RV visualized in PET/MRI, and both sTWEAK and IL-6 predict clinical deterioration.

Key words: pulmonary arterial hypertension, IL-6, imaging, PET, sTWEAK

Background

Pulmonary arterial hypertension (PAH) is a multifactorial disease characterized by proliferation and vasoconstriction in pulmonary vasculature. These lead to subsequent increase of pulmonary vascular resistance (PVR), right-sided heart failure (HF) and premature death.[1] The development of PAH involves inflammatory processes together with in situ thrombosis, where circulating cytokines (also those released by platelets at the site of endothelium injury) play an important role.[2–5] Some studies revealed that not only the excess of platelet-derived cytokines, but also their deficiency may be associated to PAH and its progression, and thus platelet blockade may not be a correct action to treat PAH.[6–8] Platelets are one of the main sources of circulating tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and we have shown that it is platelet TWEAK concentration that provides prognostic information in PAH.[6] Our previous studies have proven that PAH patients present lower serum and platelet lysate soluble TWEAK (sTWEAK) concentrations than the control group, which is also associated with a worse prognosis. However, possible mechanism resulting in lower sTWEAK in these patients as well as its involvement in the pathogenesis of the disease are still unclear.

Interleukin 6 (IL-6) is a pleiotropic cytokine involved in acute phase of inflammation, which acts via many receptors, e.g., CD126, gp130 (also called CD130) or the soluble form of IL-6 receptor (sIL-6R).[9] In our previous project, we have demonstrated that patients with PAH have higher concentrations of serum and plasma IL-6 together with sIL-6R than healthy volunteers, with no significant differences in soluble gp130 (sgp130) levels, which strongly indicates enhanced IL-6 trans-signaling in patients with PAH.[5] Unlike sTWEAK, IL-6 plasma concentration is more important in PAH than its platelet content.[10] There is evidence for an important role of IL-6 in lipid cardiac metabolism.[11,12]

Physiologically, most (even 95%) of energy in cardiomyocytes is derived from phosphorylation processes occurring in mitochondria (predominantly from fatty acids and, to a lesser extent, carbohydrate metabolism), with the remainder coming from process of glycolysis.[13–15] Interleukin 6 is involved in maintaining the balance between fatty acid oxidation and cardiac lipotoxicity, where its deficiency results in intracellular toxic lipid accumulation, thereby precipitating mitochondrial oxidative phosphorylation and thus overall cardiac dysfunction.[12,16]

However, no previous studies focused on the possible link between the levels of IL-6 or sTWEAK and quantitative measure of altered cardiac metabolism in case of HF. In PAH, increased PVR and following right ventricle (RV) pressure overload is associated with higher ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake in positron emission tomography (PET) imaging.[17,18] The newly introduced quantitative measurement of RV ^{18}F-FDG uptake, presented as standardized uptake value $(SUV)_{RV/LV}$, correlates with PAH progression and unfavorable outcomes.[19]

Objectives

The main aim of this study was to assess whether the concentrations of inflammatory cytokines are linked with metabolic and functional changes of RV, as assessed with PET/magnetic resonance imaging (MRI) in PAH patients. We also hypothesized that the alterations presented above may precede clinical deterioration in almost 5-year follow-up.

Materials and methods

Setting and participants

We enrolled 18 clinically stable adult patients diagnosed with PAH into our pilot study. The diagnosis of PAH was made using right heart catheterization (RHC) according to the European guidelines.[1] The control group consisted of 10 healthy controls who were matched based on sex and age (44.75 ±13.5 years). The exclusion criteria as well as the examination protocol were described previously.[18,19]

The clinical follow-up lasted 58 months. Death, World Health Organization (WHO) class worsening, hospitalization due to PAH, or right-sided HF were used as combined endpoint (CEP) for Kaplan–Meier analysis.

The study was approved by the Bioethics Commitee of Medical University of Bialystok, Poland (approval No. R-I-002/140/2015) and is compliant with the declaration of Helsinki.

Blood sampling

Fasting peripheral venous blood samples were obtained from patients with PAH as well as controls. Ethylenediaminetetraacetic acid (EDTA) plasma aliquots of 1.5 mL were stored at –80°C. Concentrations of plasma sTWEAK, IL-6 and sIL-6R were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. The mean minimum detectable value was 9.7 pg/mL for sTWEAK, 0.039 pg/mL for IL-6 and 6.5 pg/mL for sIL-6R. All analyses were performed according to the baseline values of the measured cytokines. Platelets were processed as described previously.[6,10] To better present platelet storage of TWEAK, IL-6 and sIL-6R, independently of the platelet count, the amounts of these cytokines were normalized to the concentration of protein in the platelet lysates and presented as a ratio in the manuscript.

Myocardial PET/MRI hybrid imaging

The PET/MRI hybrid imaging was performed with a 3T Biograph mMR hybrid system (Siemens Healthcare, Erlangen, Germany) at baseline visit within a median of 4 (2–6) days of RHC during the same patients'

hemodynamic status, according to the previously described protocol.[18–20]

For semi-quantitative analysis of ^{18}F-FDG metabolism in the myocardium, the ratio of standardized uptake value of glucose of RV and left ventricle (LV) was used ($SUV_{RV/LV}$). The late gadolinium enhancement mass index (LGEMI = LGE mass/body surface area) parameter and RV-arterial coupling estimation parameter (RV global longitudinal strain/systolic pulmonary artery pressure (RV GLS/sPAP) ratio) were assessed as described in previous studies.[18,21]

Statistical analyses

The distribution of all variables was verified using the Shapiro–Wilk test. The data are expressed as a mean standard deviation (SD) or median (interquartile range (IQR)). Statistical analysis was performed using Student's t or Mann–Whitney tests for continuous data. The Spearman's correlation coefficient was used to examine the relationship between 2 continuous variables. The Benjamini–Hochberg correction was used to account for multiple comparisons in correlation analysis. Logistic regression was used to predict a dependent categorical target variable. Univariable Cox proportional hazards regression analyses were performed to identify independent variables associated with endpoint. Receiver operator characteristic (ROC) curves were plotted to determine the area under the curve (AUC) and sensitivity and specificity of the optimal cutoffs (binomial method). To investigate the occurrence of clinical endpoints Kaplan–Meier method with log-rank test was implemented. A value of $p < 0.05$ was deemed statistically significant. A statistical software package Stata v. 13 (StataCorp LLC, College Station, USA) was used for the analysis.

Results

General results

Most PAH patients were in WHO class III (77%, n = 14). Mean pulmonary artery pressure (mPAP) derived from RHC was 50.33 ±18.96 mm Hg and PVR was 9.23 ±5.74 Wood units. Idiopathic PAH etiology was diagnosed in 64% (n = 12) of patients, PAH associated with connective tissue diseases (systemic scleroderma and mixed connective tissue disease) in 18% (n = 3) and congenital heart diseases with small left–right defects in 18% (n = 3). At the time of enrolment, 5 patients (27%) were incident cases. The clinical, functional and hemodynamic characteristics as well as laboratory data and echo results of subjects are presented in Table 1.

The PAH patients had statistically significantly lower platelet content of sTWEAK/total protein ratio than the healthy controls ($6.19 \pm 2.72 \times 10^{-8}$ compared to $9.77 \pm 2.21 \times 10^{-8}$, p = 0.006, Fig. 1A), with no significant differences in plasma levels (537.4 ±153.3 pg/mL compared to 577.5 ±59.3 pg/mL, p = 0.383). Interleukin 6 and sIL-6R plasma concentrations were significantly higher in PAH group (2.25 ±1.50 pg/mL compared to 0.82 ±0.38 pg/mL, p = 0.023 (Fig. 1B) and 45.45 ±12.11 ng/mL compared to 31.97 ±74.23 ng/mL, p = 0.004, respectively). In platelets lysate, levels of sIL-6R (normalized to total protein concentration) were significantly higher in PAH patients than in the control group (Table 1).

As reported previously,[19] mean standard uptake value of right ventricle to left ventricle ($SUV_{RV/LV}$) was higher in the PAH group (Table 1) and strongly correlated with RHC/MRI parameters of RV (Table 2).

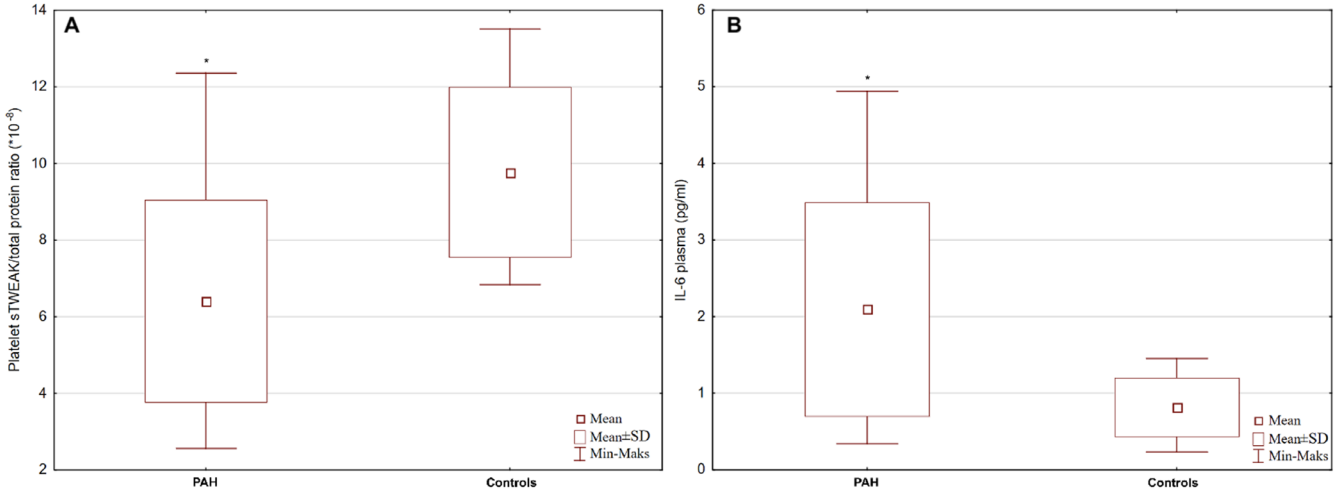

Fig. 1. sTWEAK/total protein concentrations ratio ($\times 10^{-8}$) in platelet lysate was significantly lower in PAH patients than in the control group (p = 0.006). A. IL-6 plasma concentration was significantly higher in PAH patients than in controls (p = 0.023); B. Student's t-test was used to compare 2 variables

PAH – pulmonary arterial hypertension; sTWEAK – soluble tumor necrosis factor-like weak inducer of apoptosis; SD – standard deviation; IL-6 – interleukin 6; * p-value < 0.05.

Table 1. Basic characteristics of pulmonary arterial hypertension (PAH) group and healthy controls

Variables	PAH	Controls	p-value
Subjects, n	18	10	N/A
Age [years]	48.44 ±16.71	44.75 ±13.51	0.262
Females, % (n)	72 (13)	60 (6)	N/A
6MWT distance [m]	380 ±114	N/A	N/A
BNP [pg/mL]	212 (IQR 46–335)	N/A	N/A
Idiopathic/heritable pulmonary arterial hypertension, % (n)	64 (12)	N/A	N/A
Connective tissue disease related to pulmonary arterial hypertension, % (n)	18 (3)	N/A	N/A
Congenital heart disease related to pulmonary arterial hypertension, % (n)	18 (3)	N/A	N/A
Phosphodiesterase type 5 inhibitors, % (n)	50 (9)	N/A	N/A
Endothelin receptor antagonists, % (n)	18 (3)	N/A	N/A
Phosphodiesterase type 5 inhibitors + endothelin receptor antagonists, % (n)	18 (3)	N/A	N/A
Hemodynamics			
Systemic pulmonary artery pressure [mm Hg]	79.6 ±30.7	N/A	N/A
Diastolic pulmonary artery pressure [mm Hg]	33.6 ±14.8	N/A	N/A
Mean pulmonary artery pressure [mm Hg]	50.33 ±18.95	N/A	N/A
Pulmonary capillary wedge pressure [mm Hg]	10.72 ±2.57	N/A	N/A
Diastolic pulmonary gradient [mm Hg]	23.41 ±14.01	N/A	N/A
Pulmonary vascular resistance [Wood units]	9.22 ±5.74	N/A	N/A
Cardiac index [L/min/m^2]	2.51 ±0.59	N/A	N/A
Right atrium pressure [mm Hg]	8.66 ±3.21	N/A	N/A
RV parameters (MRI)			
RV ejection fraction, %	43.99 ±9.2	63.81 ±5.81	<0.001
RV EDV/BSA [mL/m^2]	121.4 ±32.7	73.65 ±12.21	0.001
RV ESV/BSA [mL/m^2]	69.3 ±27.7	28.25 ±9.06	<0.001
RV mass/BSA [g/m^2]	42.75 ±18.82	23.8 ±4.9	<0.001
RV GLS/sPAP [%/mm Hg]	–0.26 ±0.19	N/A	N/A
LGEMI [g/m^2]	3.06 ±2.33	N/A	N/A
Myocardial metabolism (PET)			
$SUV_{RV/LV}$ ratio	1.23 ±0.86	0.19 ±0.08	<0.001
Cytokines			
sTWEAK, plasma [pg/mL]	537.4 ±153.7	577.8 ±59.7	0.383
sTWEAK/total protein, platelets ($\times 10^{-8}$)	6.19 ±2.72	9.77 ±2.21	0.006
IL-6, plasma [pg/mL]	2.25 ±1.50	0.82 ±0.38	0.023
IL-6/total protein, platelets ($\times 10^{-10}$)	0.62 ±0.51	0.50 ±0.25	0.912
sIL-6R, plasma [ng/mL]	45.45 ±12	31.97 ±7.43	0.004
sIL-6R/total protein, platelets ($\times 10^{-7}$)	1.79 ±0.61	1.31 ±0.23	0.053

Data are presented as mean ± standard deviation (SD) (normal distribution; Student's t-test was used to compare 2 variables) or median (interquartile range (IQR)) (non-normal distribution; Mann–Whitney test was used to compare 2 variables). 6MWT – 6-minute walk test; BSA – body surface area; BNP – brain natriuretic peptide; EDV – end-diastolic volume; ESV – end-systolic volume; GLS – global longitudinal strain; IL-6 – interleukin 6; LGEMI – late gadolinium enhancement mass index; LV – left ventricle; MRI – magnetic resonance imaging; PAH – pulmonary arterial hypertension; PET – positron emission tomography; RV – right ventricle; sIL-6R – soluble interleukin 6 receptor; sPAP – systolic pulmonary artery pressure; sTWEAK – soluble tumor necrosis factor-like weak inducer of apoptosis; SUV – standardized uptake value; N/A – not applicable.

Cytokines and hemodynamics interactions

We observed statistically significant correlations between platelet sTWEAK levels and PET $SUV_{RV/LV}$ (r = –0.57, p = 0.011, Fig. 2A), and between plasma IL-6 levels and $SUV_{RV/LV}$ (r = 0.50, p = 0.032, Fig. 2B). Importantly, plasma IL-6 concentration did not correlate with established hemodynamic parameters of RV dysfunction – RV ejection

Table 2. Spearman's correlations between cytokines and PET/MRI/RHC-derived parameters

Correlation coefficient (r); p-value (p)	sTWEAK, platelets	IL-6, plasma	$SUV_{RV/LV}$
6MWT distance	r = 0.08; p = 0.723	r = –0.51; p = 0.046	r = –0.38; p = 0.113
BNP	r = –0.09; p = 0.714	r = 0.18; p = 0.552	r = 0.44; p = 0.062
RVEF	r = 0.56; p = 0.019^	r = –0.10; p = 0.672	r = –0.52; p = 0.024^
mPAP	r = –0.43; p = 0.048^	r = 0.45; p = 0.051	r = 0.78; p < 0.001^
PVR	r = –0.24; p = 0.312	r = 0.22; p = 0.362	r = 0.62; p = 0.007^
$SUV_{RV/LV}$	r = –0.57; p = 0.011^	r = 0.50; p = 0.032^	–

^ p-value significant (lower than 0.05) after Benjamini–Hochberg correction; 6MWT – 6-minute walk test; BNP – serum brain natriuretic peptide; IL-6 – interleukin 6; LV – left ventricle; mPAP – mean pulmonary arterial hypertension; MRI – magnetic resonance imaging; PET – positron emission tomography; PVR – pulmonary vascular resistance; RHC – right heart catheterization; RV – right ventricle; RVEF – right ventricle ejection fraction; sTWEAK – soluble tumor necrosis factor-like weak inducer of apoptosis; SUV – standardized uptake value.

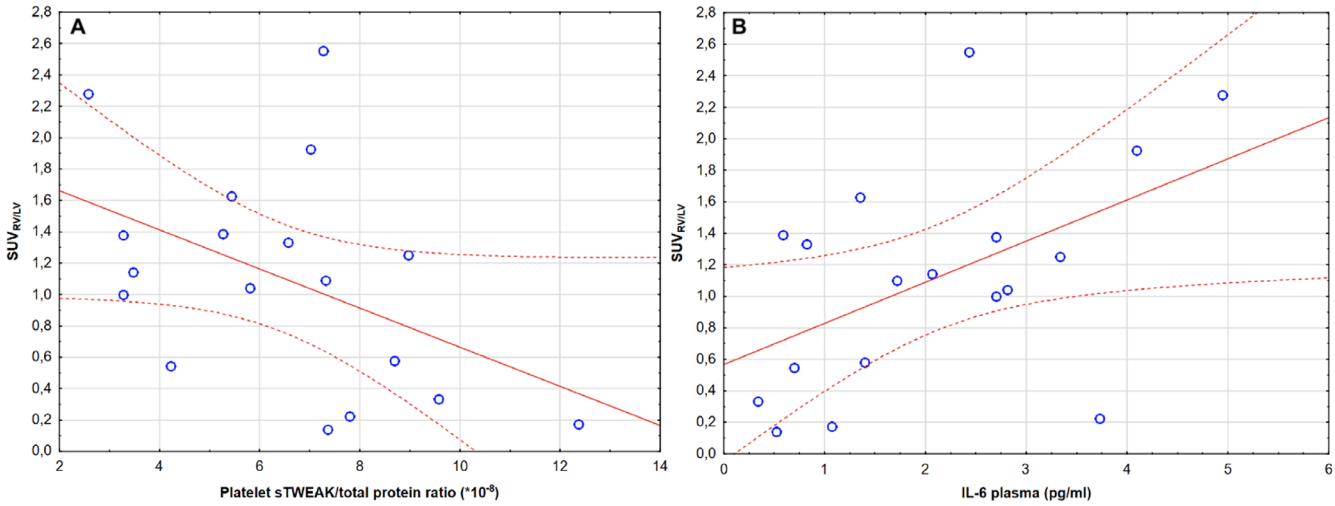

Fig. 2. Spearman's correlations between mean $SUV_{RV/LV}$ ratio and (A) sTWEAK platelet content (r = –0.57, p = 0.011) and (B) IL-6 plasma concentration (r = 0.50, p = 0.032)

SUV – standardized uptake value; RV – right ventricle; LV – left ventricle; PAH – pulmonary arterial hypertension; sTWEAK – soluble tumor necrosis factor-like weak inducer of apoptosis; IL-6 – interleukin 6.

fraction (RVEF), PVR, mPAP, or cardiac index, but it was a predictor of clinically significant $SUV_{RV/LV}$ threshold >1 in the univariate logistic regression analysis (hazard ratio (HR) = 3.62, 95% confidence interval (95% CI): [1.21; 10.17], p = 0.011). Also, platelet sTWEAK was a predictor of $SUV_{RV/LV}$ > 1 (HR = 0.44, 95% CI: [0.23; 0.84, p = 0.017).

In logistic regression, platelet sTWEAK was a predictor of RVEF < 40% (related with worse prognosis[19]; HR = 0.58, 95% CI: [0.33; 1.12], p = 0.048) and of newly established RV dysfunction and PAH prognostic parameter – RV GLS/sPAP > (–)0.29 (HR = 0.46, 95% CI: [0.2; 1.08], p = 0.043). Furthermore, we observed that platelet sTWEAK correlated with MRI and RHC RV parameters – RVEF (r = 0.56, p = 0.019) and mPAP (r = –0.43, p = 0.048), which proves a possible strong relationship between its altered availability and PAH hemodynamics.

Interestingly, plasma IL-6 predicted LGEMI parameter (LGE mass in RV insertion points normalized to body surface area) higher than 2.75 (cutoff value of worse PAH prognosis[21]; HR = 2.32, 95% CI: [0.93; 5.81], p = 0.046).

Survival analysis

We analyzed the concentrations of cytokines in PAH subjects who had met CEP (72%, n = 13). In this study, we extended the follow-up to 58 months. During this period, 4 patients died and 9 patients experienced a WHO class worsening with hospitalization (including 5 patients who required initiation of parenteral prostacyclin analogue). Mean time to clinical worsening was 25.38 ±18.58 months.

Patients with CEP had lower baseline platelet sTWEAK ($5.39 \pm 2.38 \times 10^{-8}$ compared to $8.30 \pm 2.60 \times 10^{-8}$, p = 0.032) and higher plasma IL-6 (2.55 ±1.51 pg/mL compared to 1.46 ±0.99 pg/mL, p = 0.041) than stable patients. Furthermore, CEP group presented higher $SUV_{RV/LV}$ ratio together with MRI parameter – RVEF and RHC parameters – mPAP and RAP, which is consistent with our previous results (Table 3).[18,19]

Using ROC curve analysis, we determined cutoff value of sTWEAK/total protein concentration ratio in platelets in prediction of CEP. Patients with a value lower than

Table 3. Comparison of patients with combined endpoint (CEP) and without CEP

Variables	CEP (+) patients	CEP (–) patients	p-value
Patients, n	13	5	N/A
BNP [pg/mL]	252 (IQR 143–507)	146 (IQR 46–252)	0.092
6MWT distance [m]	344 ±108	477 ±70	0.032
$SUV_{RV/LV}$ ratio	1.50 ±0.82	0.64 ±0.49	0.023
RVEF [%]	41.68 ±8.54	48.87 ±8.57	0.094
mPAP [mm Hg]	57.69 ±16.29	31.83 ±9.38	0.002
PVR [Wood units]	11.22 ±5.37	4.38 ±2.08	0.006
RAP [mm Hg]	9.69 ±3.13	6.12 ±1.26	0.013
RV GLS/sPAP [%/mm Hg]	–0.19 ±0.14	–0.47 ±0.19	0.024
LGEMI [g/m^2]	3.81 ±2.12	0.67 ±0.19	0.016
sTWEAK, plasma [pg/mL]	538.7 ±169.7	535.8 ±145.4	0.605
sTWEAK/total protein, platelets ($\times 10^{-8}$)	5.39 ±2.38	8.30 ±2.60	0.032
IL-6, plasma [pg/mL]	2.55 ±1.51	1.46 ±0.99	0.041
IL-6/total protein, platelets ($\times 10^{-10}$)	0.64 ±0.38	0.58 ±0.65	0.794
sIL-6R, plasma [ng/mL]	44.41 ±9.36	46.95 ±15.7	0.885
sIL-6R/total protein, platelets ($\times 10^{-7}$)	1.49 ±0.46	2.19 ±0.57	0.086

Data are presented as mean ± standard deviation (SD) (normal distribution; Student's t-test was used to compare 2 variables) or median (interquartile range (IQR)) (non-normal distribution; Mann–Whitney test was used to compare 2 variables). BNP – brain natriuretic peptide; CO – cardiac output; GLS – global longitudinal strain; IL-6 – interleukin 6; LGEMI – late gadolinium enhancement mass index; LV – left ventricle; mPAP – mean pulmonary artery pressure; PAC – pulmonary arterial compliance; PVR – pulmonary vascular resistance; RAP – right atrial pressure; RV – right ventricle; RVEF – right ventricle ejection fraction; sIL-6R – soluble interleukin 6 receptor; sPAP – systolic pulmonary artery pressure; sTWEAK – soluble tumor necrosis factor-like weak inducer of apoptosis; SUV – standardized uptake value; 6MWD – 6-minute walk test; N/A – not applicable.

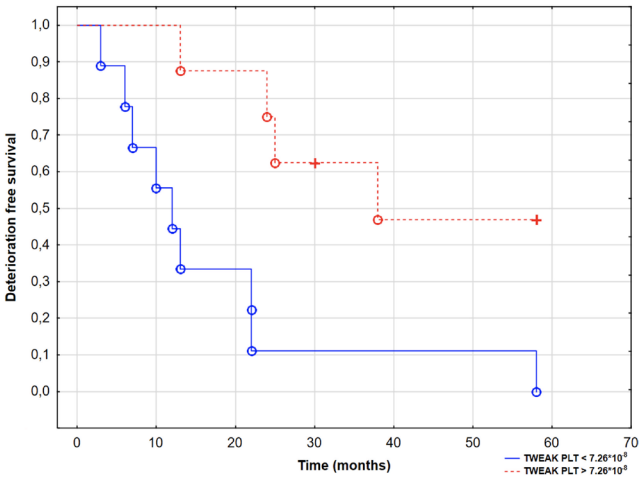

Fig. 3. Kaplan–Meier curves presenting deterioration-free survival in patients with pulmonary arterial hypertension (PAH) based on soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) platelet lysate content, log-rank test, p = 0.006

° – complete events; + – censored events.

7.26×10^{-8} (AUC = 0.78 (95% CI: [0.66; 0.96]), p = 0.021) had worse prognosis; the Kaplan–Meier survival curve and the log-rank test (p = 0.006) are presented in Fig. 3.

Interestingly, the same cutoff value of platelet sTWEAK/total protein concentration ratio was obtained in prediction of $SUV_{RV/LV}$ ratio higher than 1 in PAH patients (AUC = 0.89 (95% CI: [0.76; 1]), p < 0.001). This suggests that advanced metabolic changes in PAH patients are in strong relation with cytokine levels affecting prognosis.

Finally, univariate Cox proportional hazard analysis revealed that among various parameters, platelet sTWEAK and plasma IL-6 together with RV GLS/sPAP, RVEF, mPAP, and $SUV_{RV/LV}$ were significantly associated with time to CEP (Table 4).

Discussion

In physiological conditions, cardiomyocytes use mainly fatty acid oxidation to produce energy. It changes in case of HF (e.g., PAH) or during ischemia.[17,20] Our previous results suggest that in PAH, possible "metabolic shift" occurs and process of glycolysis is highly increased, especially in RV, which can be assessed with PET imaging.[19] With further PAH progression and RV uncoupling, RV ^{18}F-FDG uptake increases and finally surpasses LV uptake ($SUV_{RV/LV}$ > 1). Importantly, $SUV_{RV/LV}$ ratio may independently predict PAH patients' prognosis preceding significant clinical deterioration requiring hospitalization.[14,19] On the other hand, RV ^{18}F-FDG accumulation may decrease after specific treatment with epoprostenol, proportionally to the degree of reduction in the PVR and RV peak-systolic wall stress.[22]

Next, we were able to confirm that patients with PAH have altered plasma IL-6 concentration together with sIL-6R and diminished platelet sTWEAK content.[6,10] These

Table 4. Univariate Cox proportional hazard analysis for the time to clinical worsening

Value	p-value	HR (95% CI)
Age	0.592	0.99 [0.93; 1.06]
WHO class	0.092	4.52 [0.7; 29.1]
BNP	0.121	1.00 [0.99; 1.02]
6MWT distance	0.062	0.99 [0.98; 1]
Creatinine	0.634	1.12 [0.71; 1.71]
LVEF	0.232	1.05 [0.96; 1.15]
RV EDV/BSA	0.476	1.01 [0.98; 1.03]
RV ESV/BSA	0.113	1.03 [0.99; 1.06]
RV mass/BSA	0.082	1.05 [0.99; 1.11]
RVEF	0.019	0.86 [0.71; 0.95]
mPAP	0.018	1.15 [1.01; 1.31]
PCWP	0.102	1.38 [0.93; 2.02]
DPG	0.068	1.07 [0.99; 1.14]
RAP	0.013	1.61 [1.08; 2.41]
Cardiac index	0.302	0.47 [0.11; 1.95]
PVR	0.048	1.44 [1.07; 1.94]
$SUV_{RV/LV}$	0.017	7.01 [2.24; 19.23]
RV GLS/sPAP	0.019	18.94 [2.23; 28.32]
IL-6, plasma	0.192	1.77 [0.69; 4.54]
sTWEAK, platelet	0.048	0.61 [0.34; 0.92]

HR – hazard ratio; 95% CI – 95% confidence interval; BSA – body surface area; BNP – brain natriuretic peptide; EDV – end-diastolic volume; ESV – end-systolic volume; DPG – diastolic pulmonary gradient; GLS – global longitudinal strain; LGEMI – late gadolinium enhancement mass index; LV – left ventricle; LVEF – left ventricle ejection fraction; mPAP – mean pulmonary artery pressure; sPAP – systolic pulmonary artery pressure; PCWP – pulmonary capillary wedge pressure; PAH – pulmonary arterial hypertension; PVR – pulmonary vascular resistance; RAP – right atrial pressure; RV – right ventricle; RVEF – right ventricle ejection fraction; sTWEAK – soluble tumor necrosis factor-like weak inducer of apoptosis; SUV – standardized uptake value; WHO – World Health Organization; IL-6 – interleukin 6; 6MWT – 6-minute walk test

results are in line with earlier observations. In this study, we have reported for the first time that the levels of these cytokines are also strongly correlated with altered glucose metabolism of cardiomyocytes. Furthermore, we were able to obtain prognostic cutoff value of sTWEAK platelet level, which may help in risk assessment of PAH patients.

Endothelial dysfunction and in situ thrombosis are considered important pathological mechanisms in PAH.[23] Activated platelets release various growth factors and cytokines enhance vasoconstriction and thrombus formation.[24] Platelet-derived mediators can not only initiate or aggravate local vascular remodelling but also attenuate it; therefore, simple platelet inhibition may not be appropriate in PAH. The sTWEAK is described as "healing cytokine" which promotes tissue regeneration or regional healing of pulmonary vasculature.[7] Platelet lysate concentration of this cytokine was linked in our study with worse prognosis in PAH patients, so it seems that local availability of sTWEAK at the site of endothelium injury is very important and may differ from the amount released during platelet activation.

Interleukin 6 is an important cytokine in PAH pathogenesis.[2,25] Plasma or serum IL-6 levels are elevated both in patients with PAH and in animal models of PAH, and have prognostic value.[10,26] New evidence suggests that IL-6 and sIL-6R play an important role in lipid metabolism.[12] They not only play an anti-obesity role in rodent metabolic homeostasis, but also increase fatty acids oxidation, e.g., in human cardiomyocytes.[16] The IL-6 provides precise balance between fatty acid oxidation and lipotoxicity (excess of fatty acids) in cardiomyocytes, preventing cellular injury (e.g., mitochondria dysfunction, endoplasmic reticulum (ER) stress) and cardiac energy deficit.[27]

As we described before, cardiac metabolism is altered in PAH.[19] The strong relationship between pressure and/or volume overload of RV and enhanced glycolysis in cardiomyocytes is confirmed; however, there are still many questions about the sequence of pathophysiological events underlying this phenomenon. It is still not clear whether "metabolic shift" in RV is secondary to hemodynamic impairment or it results from strongly altered inflammatory processes occurring in PAH (probably the inflammatory processes in PAH are not only limited to pulmonary vasculature). In our results, both platelet sTWEAK and plasma IL-6 content were statistically significantly correlated with glucose uptake measured in PET imaging. The exact mechanism of these interactions is still not clear, but this study provides insight into difficult and multifactorial PAH pathogenesis. We believe that research on the significance of cardiac metabolism and its alterations may be an important step in developing potential therapies based on metabolic modulations.

Furthermore, our previous results considering sTWEAK platelet content and $SUV_{RV/LV}$ have a significant prognostic value which can help in risk assessment and therapy plans of already seriously ill PAH patients. In this study, we presented prolonged observation (up to 5 years) and partially validated prognostic significance of previously discussed parameters.[6,10]

Limitations

We are aware of several limitations of our research. The study group was relatively small (but homogeneous) and consisted of mostly advanced PAH patients. The PET/MRI is still an expensive imaging method and thus the promising results presented above may encourage to undertake a similar research on a larger group of patients. Next, patients were on various approved treatments for PAH, often in combination, and we were not able to assess the impact of disease-modifying therapy on the concentrations of cytokines. Due to small cohort, we also did not perform a multivariate analysis of factors independently affecting clinical deterioration. Additional studies in larger patient cohorts, aimed at establishing the reliability and overall usefulness of presented analysis in predicting survival, are now required.

Conclusions

The sTWEAK and IL-6 concentrations in PAH patients are linked with metabolic and functional changes of RV visualized in PET/MRI imaging and occurring before clinical deterioration. This may suggest that RV dysfunction in PAH is caused not only by simple pressure and/or volume overload due to pulmonary vasoconstriction, but also results from complex pathogenesis. The interplay between platelets, circulating cytokines and cardiac metabolism may help understand PAH pathogenesis, but further significance of these phenomena should be investigated in prospective studies.

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