Research Article

Pulmonary Circulation

Higher plasma fibroblast growth factor 23 levels are associated with a higher risk profile in pulmonary arterial hypertension

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Abstract

Metabolic abnormalities are proposed to contribute to pulmonary arterial as well as right ventricular remodelling in pulmonary arterial hypertension. Among the proposed abnormalities are altered glucose and lipid processing, mitochondrial malfunction, oxidative stress as well as vitamin D and iron abnormalities. In the present study, we investigated 11 metabolic plasma biomarkers, with the hypothesis that metabolic proteins may mirror disease severity in pulmonary arterial hypertension. Using proximity extension assays, plasma metabolic biomarkers were measured in 48 pulmonary arterial hypertension patients at diagnosis and, in 33 of them, at an early treatment follow-up, as well as in 16 healthy controls. Among the studied metabolic biomarkers, plasma fibroblast growth factor-23 ($p < 0.001$), fibroblast growth factor-21 ($p < 0.001$), fatty acid binding protein 4 ($p < 0.001$) and lectin-like oxidised low-density lipoprotein receptor 1 ($p < 0.001$) were increased and paraoxonase-3 was decreased ($p < 0.001$) in pulmonary arterial hypertension at diagnosis versus controls. Fibroblast growth factor-23 showed the strongest correlations to studied clinical parameters and was therefore selected for further analyses. Fibroblast growth factor-23 correlated specifically to mean right atrial pressure ($r = 0.67$, $p < 0.001$), six-min walking distance ($r = -0.66$, $p < 0.001$), NT-proBNP ($r = 0.64$, $p < 0.001$), venous oxygen saturation ($r = -0.61$, $p < 0.001$), cardiac index ($r = -0.39$, $p < 0.007$) and pulmonary vascular resistance ($r = 0.37$, $p < 0.01$). Fibroblast growth factor-23 correlated moreover to ESC/ERS ($r = 0.72$, $p < 0.001$) and the REVEAL risk score ($r = 0.61$, $p < 0.001$). Comparing early treatment follow-up with baseline, fibroblast growth factor-23 decreased ($p < 0.02$), with changes in fibroblast growth factor-23 correlating to changes in six-min walking distance ($r = -0.56$, $p < 0.003$), venous oxygen saturation ($r = -0.46$, $p < 0.01$), pulmonary vascular resistance ($r = 0.43$, $p < 0.02$), mean right atrial pressure ($r = 0.38$, $p < 0.04$) and cardiac index ($r = -0.39$, $p < 0.04$). Elevated plasma fibroblast growth factor-23 levels at pulmonary arterial hypertension diagnosis were associated with worse haemodynamics and a higher risk profile, and were decreased after the administration of pulmonary arterial hypertension-specific treatment.

Keywords

pulmonary arterial hypertension, risk assessment, metabolism, fibroblast growth factor 23

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Introduction

In pulmonary arterial hypertension (PAH), distal pulmonary arteries manifest with intimal thickening and fibrosis, medial hypertrophy and complex lesion formation, resulting in high pulmonary vascular pressures and declined right ventricular function.[1] Although the cellular mechanisms causing these vascular changes are not completely understood, new theories for PAH pathogenesis have emerged

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during the last decade. Among these is a metabolic theory that describes the negative impact of abnormal glucose and lipid processing, mitochondrial dysfunction, oxidative stress as well as iron and vitamin D deficiencies on PAH.[2–9]

Malignant tumours manifest with a metabolic shift to increased glucose uptake, aerobic glycolysis and lactate production; which favour carcinogenesis.[10] Similar processes are observed in PAH, where they are suggested to promote pulmonary arterial remodelling.[9] Pulmonary artery endothelial cells from patients with PAH exhibit an increased glycolytic rate, and positron emission tomography scans show that glucose uptake is increased in human PAH lungs.[2,3] Using dichloroacetate to shift intracellular metabolism from glycolysis to increased glucose oxidation, both prevents and reverses PAH in monocrotaline models.[4,5] In addition to the altered glucose processing, a disturbed lipid metabolism could also fuel PAH progression. As discussed in a review from Sharma and colleagues, oxidised lipids contribute to numerous PAH features, including smooth muscle cell proliferation and inflammation.[8] In recent years, there has been a growing interest for metabolites in PAH. In lungs from PAH patients, intermediates in various metabolic processes, including glycolysis and the tricarboxylic acid cycle (TCA), have been shown to be disturbed.[11] In plasma, many metabolites' levels are abnormal in PAH patients, including TCA intermediaries, purine metabolites, tryptophan breakdown products, fatty acids, sphingomyelins and steroids.[12,13]

In the present study, we measured new plasma metabolic biomarkers, in treatment-naïve PAH patients, in relation to haemodynamics, treatment response, as well as risk stratification according to the REVEAL risk score and the current European Society of Cardiology/European Respiratory Society (ESC/ERS) PAH guidelines.[14,15] We hypothesised that some of these metabolic biomarkers may mirror disease severity and risk status in patients with PAH.

Method

Study population

The present study included 48 adult, treatment-naïve patients with PAH who were diagnosed through right heart catheterisation, between September 2011 and September 2016, at the Hemodynamic Lab at Skåne University Hospital in Lund, Sweden. Patients were diagnosed according to the, at the time, current ESC/ERS 2009 or 2015 PAH guidelines.[16,17] Twenty-one patients had idiopathic and two had familial PAH. These were treated as an entity referred to as IPAH/FPAH. Twenty-one systemic sclerosis-associated PAH and four other connective tissue disease-associated PAH patients were pooled into another entity, referred to as CTD-PAH.

Plasma sampling

During baseline right heart catheterisations, venous blood was obtained from all PAH patients through the introducer in the internal jugular vein. Blood samples at an early treatment follow-up, 116 (90–127) days after PAH treatment initiation, were available for 33 of the patients for further analyses. Venous blood was additionally obtained from 16 healthy control subjects. Plasma was extracted and stored at –80°C in the Lund Cardio Pulmonary Register cohort of Region Skånes Biobank. All participating subjects were informed regarding the purpose of the collection and storage of the blood samples, and all have given their written consents. The local ethics board in Lund has approved the study (Dnr 2015/270, Dnr 2011/777, Dnr 2011/368, Dnr 2010/114, Dnr 2010/442).

Biomarker analyses

Proseek multiplex cardiovascular II and III, as well as oncology, 96-plex immunoassays (Olink Proteomics, Uppsala, Sweden) were used to analyse the present biomarkers. From these panels, metabolic biomarkers were chosen for analysis, including 2,4-dienoyl-CoA reductase, fatty acid-binding protein (FABP)-2 and -4, fibroblast growth factor (FGF)-21 and -23, low-density lipoprotein (LDL) receptor, leptin, lectin-like oxidised LDL receptor 1 (LOX-1), lipoprotein lipase, proprotein convertase subtilisin/kexin type 9 and paraoxonase-3 were assessed using proximity extension assays (PEA). PEA technique has previously been described in detail.[18] In brief, oligonucleotide-labelled antibody pairs are used to detect targeted biomarkers, in order to avoid unspecific antibody binding and potential cross-reactivity events. As two related probes are brought in close proximity, the oligonucleotides hybridise in a pair-wise manner. DNA polymerase addition results in a proximity-dependent DNA polymerisation event, creating a unique PCR target sequence. The DNA sequence is then detected and quantified using a microfluidic real-time PCR instrument (Biomark HD; Fluidigm, San Francisco, CA, USA). Data quality control and normalisation is performed utilising an internal extension control and an inter-plate control, in order to adjust for intra- and inter-run variation. Assay validation data and panel information are available at www.olink.com.

In the Proseek multiplex oncology analyses, one control sample and one patient follow-up sample did not pass analysis standards and were therefore excluded. In the cardiovascular II panel, two patient's follow-up samples were excluded for the same reason.

The biomarkers are presented as arbitrary units on a $\log_2$ scale. N-terminal prohormone of brain natriuretic peptide (NT-proBNP) values used for correlation analyses were measured using PEA. NT-proBNP values used for patient risk scores were extracted from medical journals, in order to obtain absolute biomarker concentrations.

These NT-proBNP values were analysed using electro-chemiluminescence immunoassays.

Haemodynamic assessment

Right heart catheterisations were performed using Swan-Ganz catheters. Thermodilution was used to measure cardiac output (CO). Haemodynamic, 6-min walking distance (6MWD), mixed venous oxygen saturation (SvO_2) and WHO functional class (WHO-FC) were extracted from medical records.

Body surface area (BSA), mean right atrial pressure (MRAP), mean pulmonary artery pressure (MPAP), pulmonary artery wedge pressure (PAWP), and CO were used to calculate cardiac index (CI), and pulmonary vascular resistance (PVR), according to the formulae: $CI = CO/BSA$, and $PVR = (MPAP - PAWP)/CO$.

Renal function

Relative estimated glomerular filtration rate (eGFR) was calculated based on creatinine values, using the revised Lund-Malmö GFR estimating equation, via www.egfr.se.[19]

Risk score calculations

MRAP, CI, WHO-FC, 6MWD, NT-proBNP and SvO_2 were used to calculate an ESC/ERS guideline risk score, based on the method presented by Kylhammar et al.[20] Each parameter was graded with a score from 1 to 3, according to the cut-offs provided in the risk assessment instrument from the current ESC/ERS PAH guidelines,[14] where 1 corresponded to 'low', 2 to 'intermediate', and 3 to 'high' risk.[20] A mean of the parameter scores was then calculated, which defined a patient's risk score in the present study. This risk score is referred to as 'ESC/ERS guideline risk score'. Ten patients had one missing parameter, and one patient had two missing parameters.

REVEAL risk score (1.0) was additionally calculated for all PAH patients.[15] As some patients had a few missing values, we calculated a modified score using a mean of the available points and did not include the always-assigned six points in the original algorithm. Renal insufficiency was defined as plasma creatinine $>120\,\mu mol/L$. Additional data related to the REVEAL risk score parameters used in the present study population have previously been documented as supplemental material.[21]

Analysis approach

All metabolic biomarkers were tested between PAH patients at diagnosis and controls, between IPAH/FPAH and CTD-PAH patients, as well as in PAH patients at diagnosis versus treatment follow-up. Biomarkers that showed to be different between PAH patients at diagnosis and controls were selected for correlation studies against risk assessment parameters at baseline, including MRAP, CI, SvO_2, 6MWD, plasma NT-proBNP as well as PVR. The biomarkers that showed the strongest correlations to these clinical parameters were pre-defined to be selected for correlation analyses against ESC/ERS guideline and REVEAL risk scores. Baseline plasma NT-proBNP was moreover tested for correlations against MRAP, CI, PVR, SvO_2 and 6MWD.

Biomarkers that showed to be different between PAH patients at diagnosis and treatment follow-up were additionally pre-defined to be selected for correlation analyses that test biomarker changes (Δ between baseline and follow-up) against changes in ESC/ERS guideline risk, MRAP, CI, PVR, SvO_2, 6MWD and NT-proBNP. Finally, changes in plasma NT-proBNP were moreover tested for correlations against changes in MRAP, CI, PVR, SvO_2 and 6MWD.

Statistics

Statistics were performed using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) or GraphPad Prism (Version 7, GraphPad Software, La Jolla, CA, USA).

Mann–Whitney U test was used to evaluate differences between baseline biomarker values in PAH patients versus control subjects, as well as in IPAH/FPAH versus CTD-PAH subgroups. The Benjamini-Hochberg procedure was used to assess false discovery rate (FDR) for the statistical tests comparing PAH patients to controls. FDR was set to 5%. The total number of statistical tests was 273, based on all the biomarkers included in the studied panels. Parameters were compared between baseline and follow-up with a paired t-test or a Wilcoxon signed-rank test. All correlations were assessed using Spearman's correlations.

Statistical significance was defined as $p < 0.05$. All values are presented as medians (interquartile range), unless otherwise stated.

Results

Population characteristics

Baseline and follow-up characteristics are provided in Table 1. At treatment follow-up, patients were on monotherapy with either bosentan ($n = 5$), macitentan ($n = 4$), ambrisentan ($n = 7$), sildenafil ($n = 4$) or tadalafil ($n = 2$) or a combination therapy with bosentan and sildenafil ($n = 1$), macitentan and sildenafil ($n = 2$), macitentan and tadalafil ($n = 1$), macitentan, tadalafil and treprostinil ($n = 1$), ambrisentan and sildenafil ($n = 1$) or ambrisentan and tadalafil ($n = 3$). Among these, some had nifedipin due to rheumatologic symptoms during both baseline and follow-up ($n = 9$) or only at baseline ($n = 1$). Acute vaso-reactive patients were on either nifedipin alone ($n = 1$) or nifedipin and macitentan ($n = 1$).

Table 1. Patient characteristics.

	All PAH patients[a]	PAH subset *before treatment*	PAH subset *at treatment follow-up*	CTD-PAH *before treatment*	IPAH/FPAH *before treatment*
Sample size *n* (% females)	48 (83)	33 (88)	33 (88)	25 (92)	23 (74)
Age, years	72 (64–76)	71 (61–77)	72 (61–77)	71 (65–76)	73 (57–77)
BSA m^2	1.8 (1.6–2.0)	1.7 (1.6–1.8)	1.7 (1.6–1.8)	1.7 (1.6–1.8)	1.8 (1.6–2.0)
Comorbidities n					
Hypertension	17	11	NA	5	12
Diabetes mellitus	12	8	NA	2	10
Atrial fibrillation	4	2	NA	2	2
Stroke	2	2	NA	0	2
IHD	7	5	NA	3	4
Thyroid disease	11	10	NA	6	5
WHO-FC n[d]					
I	1	1	2	0	1
II	9	6	10	6	3
III	28	22	15	12	16
IV	2	2	0	2	0
Haemodynamics					
MPAP mmHg	43 (37–54)	43 (37–55)	36 (32–48)[b]	39 (30–43)	51 (42–56)[c]
PAWP mmHg	8 (6–11)	6 (5–9)	8 (5–11)	8 (5–10)	9 (6–11.5)
MRAP mmHg	7 (4–11)	6 (3–9)	6 (3–9)	6 (3–9)	9 (6–11)[c]
CI l min^{-1} m^{-2}	2.2 (1.8–2.8)	2.3 (1.8–2.9)	2.7 (2.2–3.3)[b]	2.6 (2.0–3.1)	1.9 (1.7–2.2)[c]
PVR WU	9.5 (6.7–11.7)	9.6 (7.0–11.9)	5.8 (4.5–8.6)[b]	6.9 (4.8–9.7)	11.5 (9.2–13.5)[c]
6MWD, m[e]	242 (176–348)	242 (191–345)	270 (234–330)[b]	267 (188–352)	225 (163–278)
SvO_2 %	59 (51–66)	62 (55–66)	63 (59–72)[b]	65 (55–71)	55 (49–60)[c]
eGFR[e] mL^{-1} min^{-1} 1.73 m^2	59 (45–68)	NA	NA	62 (45–70)	57 (45–66)
Treatment (at follow-up) n					
ERA			16		
PDE5i			6		
ERA and PDE5i			8		
Triple combination			1		
CCB and ERA			1		
CCB			1		

Age, BSA, comorbidities and WHO-FC were not statistically tested.
Continuous values are presented as median (interquartile range).
[a]All PAH patients at diagnosis.
[b]Significant difference before versus after treatment in PAH patients.
[c]Significant baseline differences between IPAH/FPAH and CTD-PAH.
[d]The total number of patients with functional class values does not match the sample size due to missing values.
[e]Two missing values.
PAH: pulmonary arterial hypertension; CTD-PAH: connective tissue disease-associated PAH; IPAH: idiopathic PAH; FPAH: familial PAH; 6MWD: 6-min walk distance; BSA: body surface area; CCB: calcium channel blocker; CI: cardiac index; eGFR: estimated glomerular filtration rate; ERA: endothelin receptor antagonist; IHD: ischemic heart disease; MPAP: mean pulmonary arterial pressure; MRAP: mean right atrial pressure; NA: not assessed; PDE5i: phosphodiesterase type 5 inhibitor; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation; SVR: systemic vascular resistance; WHO-FC: World Health Organization functional class; WU: wood units.

Metabolic biomarkers levels in PAH patients at baseline

Among the studied metabolic biomarkers, plasma FGF-23 ($p < 0.001$), FGF-21 ($p < 0.001$), FABP-4 ($p < 0.001$) and LOX-1 ($p < 0.001$) were increased and paraoxonase-3 was decreased ($p < 0.001$) in the PAH patients at baseline compared to controls (Table 2).

Metabolic biomarker correlations to clinical parameters at baseline

Biomarkers that showed to be different between PAH patients and controls were selected for correlation studies at baseline. Baseline FGF-23 correlated to baseline MRAP ($r = 0.67$, $p < 0.001$), 6MWD ($r = -0.66$, $p < 0.001$), NT-proBNP

Table 2. Plasma metabolic biomarkers in pulmonary arterial hypertension patients.

	Controls	All PAH patients[a]	PAH subset *before treatment*	PAH subset *at treatment follow-up*	CTD-PAH *before treatment*	IPAH/FPAH *before treatment*
Age years	46.5 (30–51)	71.5 (64–76)	71 (60.5–76.5)	72 (61–76.5)	71 (64.5–76)	73 (57–77)
Biomarkers (AU)						
NT-proBNP	0.18 (0.06–0.2)	3.13 (2.13–3.81)[b]	2.94 (2.05–3.35)	2.08 (1.3–2.86)[c]	2.54 (1.28–3.62)	3.28 (2.96–3.82)
FGF-23	3.82 (3.56–4.01)	5.49 (4.86–6.24)[b]	5.6 (4.59–6.37)	4.74 (4.18–5.93)[c]	5.31 (4.23–6.04)	5.73 (5.34–6.43)
FGF-21	5.51 (4.99–6.09)	7.61 (6.53–8.48)[b]	6.96 (6.3–8.33)	6.98 (5.64–8.06)	6.86 (6.2–8.09)	8.09 (6.98–8.75)
LDL receptor	3.61 (3.17–4.05)	3.28 (2.9–3.85)	3.28 (2.96–3.63)	3.18 (2.7–3.76)	3.18 (2.92–3.98)	3.29 (2.89–3.59)
LOX-1	5.97 (5.74–6.26)	6.83 (6.39–7.33)[b]	6.81 (6.39–7.25)	6.53 (6.22–6.77)[c]	6.73 (6.52–7.24)	6.84 (6.39–7.39)
Lipoprotein lipase	9.95 (9.87–10.23)	9.83 (9.56–10.06)	9.82 (9.59–9.97)	9.71 (9.53–10.02)	9.93 (9.61–10.16)	9.73 (9.56–9.88)
Leptin	5.66 (4.66–6.57)	6.36 (5.53–6.79)	6.11 (5.38–6.8)	6.08 (5.44–6.41)	6.37 (5.54–6.79)	6.32 (5.38–6.84)
FABP-2	8.11 (7.74–8.52)	8.15 (7.55–8.54)	8.22 (7.62–8.52)	8.18 (7.6–8.71)	8.1 (7.64–8.5)	8.18 (7.5–8.6)
FABP-4	3.83 (3.19–4.39)	5.13 (4.75–5.85)[b]	5.16 (4.67–5.82)	4.88 (4.06–5.55)	5.13 (4.75–5.82)	5.06 (4.8–5.89)
Paraoxonase-3	5.68 (5.34–6.07)	4.56 (4.12–5.21)[b]	4.73 (3.83–5.32)	4.74 (3.96–5.41)	4.73 (3.81–5.42)	4.48 (4.22–4.81)
DECR1	6.75 (5.35–7.71)	6.16 (5.27–7.14)	5.82 (5.08–6.83)	5.7 (4.84–6.87)	6.49 (5.38–7.3)	6.09 (5.02–7.09)
PCSK9	1.73 (1.59–1.88)	1.85 (1.59–2.01)	1.92 (1.42–2.04)	1.73 (1.31–1.98)	1.87 (1.59–2.01)	1.83 (1.6–2)

[a]All PAH patients at diagnosis.
[b]Significant difference between controls and PAH patients.
[c]Significant difference before versus after treatment in PAH patients.
Values are presented as median (interquartile range).
AU: arbitrary units; DECR1: 2,4-dienoyl-CoA reductase; FABP: fatty acid binding protein; FGF: fibroblast growth factor; LDL: low-density lipoprotein; LOX-1: lectin-like oxidised LDL receptor 1; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; PCSK9: proprotein convertase subtilisin/kexin type 9; PAH: pulmonary arterial hypertension; CTD-PAH: connective tissue disease-associated PAH; IPAH: idiopathic PAH; FPAH: familial PAH.

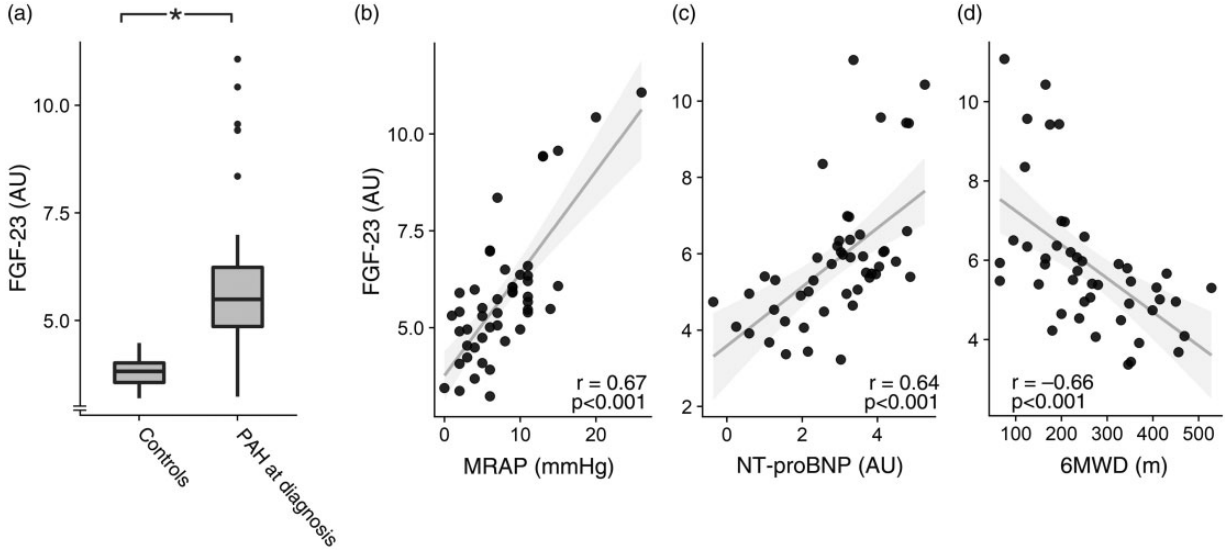

Fig. 1. Plasma fibroblast growth factor-23 (FGF-23) at diagnosis is elevated in PAH patients (a). Baseline FGF-23 correlated to mean right atrial pressure (MRAP) (b), N-terminal prohormone of brain natriuretic peptide (NT-proBNP) (c) and 6-min walking distance (6MWD) (d).
AU; arbitrary units; PAH: pulmonary arterial hypertension.
*$p < 0.05$ comparing PAH versus controls.

($r = 0.64$, $p < 0.001$) (Fig. 1), SvO_2 ($r = -0.61$, $p < 0.001$), CI ($r = -0.39$, $p < 0.007$) and PVR ($r = 0.37$, $p < 0.01$).

Moreover, baseline FGF-21 correlated to MRAP ($r = 0.51$, $p < 0.001$), CI ($r = -0.44$, $p < 0.002$), SvO_2 ($r = -0.44$, $p < 0.003$), NT-proBNP ($r = 0.37$, $p < 0.01$), PVR ($r = 0.31$, $p < 0.04$) and 6MWD ($r = -0.30$, $p < 0.05$).

Baseline FABP-4 correlated with MRAP ($r = 0.46$, $p < 0.002$), NT-proBNP ($r = 0.46$, $p < 0.002$), 6MWD ($r =$

-0.40, $p < 0.007$), SvO_2 ($r = -0.31$, $p < 0.04$) and CI ($r = -0.3$, $p < 0.04$). Baseline plasma LOX-1 correlated to SvO_2 ($r = 0.33$, $p < 0.03$), CI ($r = -0.32$, $p < 0.03$) and NT-proBNP ($r = 0.29$, $p < 0.05$). Correlations were also found between baseline paraoxonase-3 and SvO_2 ($r = 0.43$, $p < 0.003$), 6MWD ($r = 0.37$, $p < 0.02$) and MRAP ($r = -0.31$, $p < 0.03$).

Additionally, baseline NT-proBNP correlated to baseline MRAP ($r = 0.8$, $p < 0.001$), 6MWD ($r = -0.5$, $p < 0.001$), SvO_2 ($r = -0.55$, $p < 0.001$), CI ($r = -0.4$, $p < 0.005$) and PVR ($r = 0.44$, $p < 0.003$).

FGF-23 correlations to risk assessment measures at PAH baseline

Among the studied metabolic biomarkers, plasma FGF-23 at baseline was selected for correlation analyses against risk scores measures, since it showed the strongest correlations with studied clinical parameters. Plasma FGF-23 at baseline showed a correlation to both the ESC/ERS guideline risk score ($r = 0.72$, $p < 0.001$) as well as the REVEAL risk score ($r = 0.61$, $p < 0.001$) (Fig. 2).

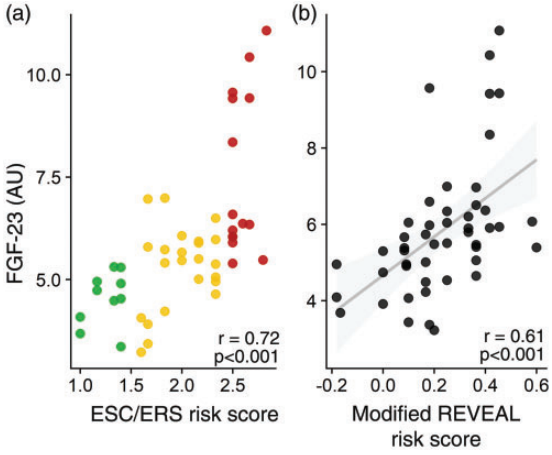

Fig. 2. Plasma fibroblast growth factor-23 (FGF-23) levels at diagnosis are correlated to risk score calculations based on the European Society of Cardiology/European Respiratory Society (ESC/ERS) PAH guidelines (a) and the REVEAL risk score (b). The dots in (a) are colour-coded: ● (green): low risk; ● (yellow): intermediate risk and ● (red): high risk.

Metabolic biomarkers between PAH baseline and treatment follow-up

Between baseline and treatment follow-up, plasma LOX-1 ($p < 0.009$) and FGF-23 ($p < 0.02$) decreased in PAH patients (Table 2). FGF-23 and LOX-1 were therefore selected for the study of correlations between biomarker changes (Δ between baseline and follow-up) and changes in selected clinical parameters.

FGF-23 changes correlated with changes in 6MWD ($r = -0.56$, $p < 0.003$), SvO_2 ($r = -0.46$, $p < 0.01$), MRAP ($r = 0.38$, $p < 0.04$) (Fig. 3), PVR ($r = 0.43$, $p < 0.02$) and CI ($r = -0.39$, $p < 0.04$). Also, changes in FGF-23 correlated with changes in ESC/ERS guideline risk status ($r = 0.47$, $p < 0.008$) (Fig. 3). LOX-1 showed no significant correlation to any studied clinical parameters.

Additionally, changes in plasma NT-proBNP correlated to changes in PVR ($r = 0.64$, $p < 0.001$), SvO_2 ($r = -0.53$, $p < 0.002$), 6MWD ($r = -0.42$, $p < 0.03$) and CI ($r = -0.39$, $p < 0.03$).

Discussion

Metabolic abnormalities are suggested to have a role in PAH pathology. The present study demonstrates that metabolic plasma biomarkers are altered in treatment-naïve PAH patients at baseline. The main results show that elevated plasma FGF-23 levels in treatment-naïve PAH patients at baseline are associated with haemodynamic severity and risk status, as assessed by the ESC/ERS guidelines and the REVEAL risk score. Moreover, FGF-23 decreased in response to PAH treatment at an early follow-up.

FGF-23, a member of the FGF family, is involved in decreasing serum phosphate levels. FGF-23 increases progressively in chronic kidney disease, presumably to counteract the phosphate accumulation seen in these patients. Nonetheless, this mineral regulator is also involved in suppressing 1-α hydroxylase, affecting vitamin D activation and calcium balance as well as parathyroid hormone expression.[22] Key inflammatory mediators, such as interleukin-6, have, furthermore, been shown to be induced in response to FGF-23.[22] Therefore, although FGF-23 was initially believed to be favourable, it is no surprise that subsequent research has identified it as a culprit protein independently linked to disease progression in chronic kidney disease.[23] Elevated FGF-23 levels have also been shown to be associated with an increased risk for heart failure and other cardiovascular diseases in chronic kidney disease patients.[24] FGF-23 directly induces cardiomyocyte hypertrophy via calcineurin-nuclear factor of activated T-cells NFAT signalling.[25] Further evidence also links FGF-23 to vasoconstriction, decreased nitric oxide availability and oxidative stress, which lead to vascular smooth muscle cell and endothelial dysfunction.[26,27]

Viewing previous knowledge, which demonstrates a direct effect of FGF-23 on the left heart and the vascular system, together with our present findings, we hypothesise that FGF-23 may be a burden to the failing right heart or the remodelled pulmonary vasculature in PAH patients. We moreover hypothesise that any such potential pathological effect may be especially important in PAH patients with concomitant kidney failure. This can be important since kidney dysfunction is common and known to increase mortality risk in PAH.[28,29] FGF-23, which is a 1-α hydroxylase regulator, may also be connected to the vitamin D deficiencies that seem to be prevalent in PAH patients.[6,7]

It is, nonetheless, important to note a previous study from Kaiser and colleagues that reported no association

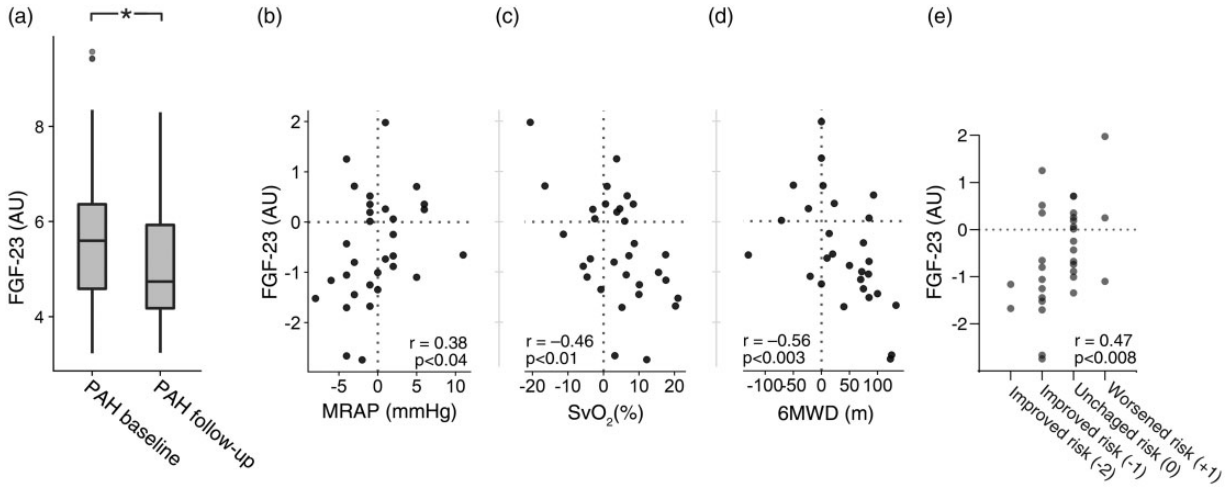

Fig. 3. Plasma fibroblast growth factor-23 (FGF-23) levels decreased after initiating PAH-specific therapy (a). Between baseline and follow-up, changes in FGF-23 (ΔFGF-23) correlated with changes in mean right atrial pressure (ΔMRAP) (b), mixed venous oxygen saturation (ΔSvO_2) (c) and 6-min walking distance (Δ6MWD) (d). FGF-23 changes also correlated to changes in risk class calculated according to the European Society of Cardiology/European Respiratory Society PAH guidelines (e). In (e), the numbers within brackets in the *x*-axis represent how many classes patients have been displaced in the risk stratification model.
AU; arbitrary units; PAH: pulmonary arterial hypertension.
*$p < 0.05$ comparing PAH at baseline versus follow-up.

between FGF-23 and mortality, in a pooled sample encompassing patients with PAH, chronic thromboembolic pulmonary hypertension and pulmonary hypertension due to lung diseases.[30] However, in this pooled sample, most patients were on PAH-specific treatment, which may partly explain the discrepancy between the present study and the investigation from Kaiser et al. In the present study population, patients at diagnosis were all treatment-naïve. Moreover, the analysis from Kaisers group were performed using an ELISA that measured the c-terminal FGF-23, whereas our measuring technique, PEA combined with PCR, uses polyclonal antibodies.

FGF-21 is another FGF family member that is involved in regulating metabolism; stimulating glucose uptake in adipocytes, increasing insulin sensitivity and lowering circulating lipids.[31] In the present study, we show that elevated random plasma FGF-21 levels in PAH patients are correlated to haemodynamics. Elevated blood FGF-21 levels are observed in diabetes mellitus type 2 patients, probably as a feedback response against the prevailing insulin resistance.[32] Interestingly, Planavila et al. have demonstrated that FGF-21 is involved in protecting the heart from oxidative stress.[33] In the field of pulmonary hypertension, Liu et al. have recently provided evidence showing that FGF-21 may improve haemodynamics and attenuate pulmonary artery smooth muscle cell proliferation in experimental hypoxia-induced pulmonary hypertension.[34]

Hyperglycaemia and insulin resistance are furthermore proposed to indirectly worsen right ventricular remodelling.[35] Notably, diabetes mellitus has been linked with an increased mortality risk in PAH.[35,36] Moreover, increased glycolysis and suppressed glucose oxidation in pulmonary arterial cells have been suggested to influence cellular growth and apoptosis in PAH, mechanisms that are described in detail in an elegant review by Sutendra et al.[9] Interestingly, malignant cells also show a shift to increased glycolysis relative to the glucose oxidation rate, which has been described as a central feature of cancer that drives proliferation and apoptosis resistance.[10] Another cornerstone in the metabolic theory described in PAH is a disturbed lipid metabolism, which is known to influence smooth muscle cell proliferation, inflammation as well as other important features in PAH.[8] In the present study, plasma FABP-4 and LOX-1 were increased whereas paraoxonase-3 was low in PAH patients compared to healthy controls. Plasma FABP-4 showed, moreover, notable correlations to MRAP and NT-proBNP. FABP-4, which is involved in lipid trafficking, is linked to obesity, insulin resistance, heart failure and other cardiovascular diseases.[37] Paraoxonase-3 is a potent antioxidant that is bound to high-density lipoproteins (HDL), and is involved in protecting LDL from oxidative modification.[38] Interestingly, low plasma HDL cholesterol is known to be associated with high mortality and clinical worsening in PAH.[39]

In recent years, there has been a growing interest for metabolites in PAH. For instance, TCA intermediaries, purine metabolites, tryptophan breakdown products, fatty acids, sphingomyelins and steroids in plasma have been shown to be abnormal in PAH patients.[12,13] The present study provides initial insight on new potential metabolic plasma biomarkers in PAH. Nonetheless, some limitations should be considered when interpreting these results. This is a retrospective study that includes a relatively small cohort, since only treatment-naïve patients were included. Moreover, having a smaller sample limited the possibility

of having a validation cohort. PAH patients included in this study are somewhat older than what is considered as the typical age for PAH. Nonetheless, the median age for PAH in Sweden is quite high (67 years) according to the Swedish PAH registry.[40] In the present analyses, we chose not to adjust the relationship between FGF-23 and ESC/ERS guideline risk score for GFR or other variables due to: (i) issues related to the statistical method assumptions and (ii) the small sample size. Having that stated, we hypothesise that any potential effects of FGF-23 on PAH may be especially important in PAH patients with concomitant kidney failure. Finally, as blood samples were taken at random timings, the possibility that blood FGF-21 levels,[41] and perhaps other metabolic biomarkers, may have been influenced by food intake should be taken into account.

Conclusion

The present study demonstrates that some metabolic biomarkers are altered, and related to haemodynamic severity, in treatment-naïve PAH patients at diagnosis. The main results show that elevated plasma FGF-23 levels in PAH patients at baseline are associated with worse haemodynamics and a higher risk profile. Plasma FGF-23 also decreases in response to PAH treatment, where changes in FGF-23 correlate to changes in ESC/ERS guideline risk score. Whether FGF-23 poses a pathological burden to the failing right heart or the remodelled pulmonary vasculature in PAH patients, and how such pathological effects would relate to renal function, are research questions that deserve further investigation in future studies.

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Conflict of interest

Habib Bouzina reports personal lecture fees from Actelion Pharmaceuticals Sweden AB. Habib Bouzina reports unrestricted research grants from the Swedish Society of Pulmonary Hypertension on behalf of GlaxoSmithKline. Roger Hesselstrand reports personal lecture fees from Actelion Pharmaceuticals Sweden AB. Göran Rådegran reports unrestricted research grants from Actelion Pharmaceuticals Sweden AB and GlaxoSmithKline, as well as personal lecture fees from Actelion Pharmaceuticals Sweden AB, GlaxoSmithKline, Bayer HealthCare AB, NordicInfu Care and Sandoz/Novatris. Göran Rådegran has been primary or co-investigator for PAH trials for Actelion Pharmaceuticals AB, GlaxoSmithKline, Pfizer, Bayer HealthCare AB and United Therapeutics.

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Contributorship

H.B., R.H. and G.R. made a substantial contribution to the concept or design of the work and/or acquisition, analysis or interpretation of data. H.B. drafted the article. H.B., R.H. and G.R. revised the manuscript critically for important intellectual content and approved the version to be published.

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