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ORIGINAL ARTICLE

Elevated plasma WIF-1 levels are associated with worse prognosis in heart failure with pulmonary hypertension

Kriss Kania[1,2]*, Abdulla Ahmed[1,2], Salaheldin Ahmed[1,2] and Göran Rådegran[1,2]

[1]*Department of Clinical Sciences Lund, Cardiology, Lund University, Lund, Sweden; and* [2]*The Haemodynamic Lab, The Section for Heart Failure and Valvular Disease, VO Heart and Lung Medicine, Skåne University Hospital, Lund, Sweden*

Abstract

Aims Heart failure (HF) is a progressive condition that is becoming more prevalent in the ageing population. Pulmonary hypertension is a common complicating factor in HF and negatively impacts survival. Plasma biomarkers are a potential method for determining the prognosis of patients with left heart failure with pulmonary hypertension (LHF-PH). We aimed to analyse the prognostic capability of 33 proteins related to, among other pathways, inflammation, coagulation, and Wnt signalling in LHF-PH.

Methods Plasma levels of 33 proteins were analysed using proximity extension assay from the plasma of 20 controls and 67 LHF-PH patients, whereof 19 underwent heart transplantation (HT). Haemodynamics in the patients were assessed using right heart catheterization.

Results Eleven proteins had elevated plasma levels in LHF-PH compared with controls ($P < 0.01$), which decreased towards the controls' levels after HT ($P < 0.01$). Survival analysis of these proteins showed that elevated plasma levels of growth hormone, programmed cell death 1 ligand 2, tissue factor pathway inhibitor 2, and Wnt inhibitory factor 1 (WIF-1) were associated with worse transplantation-free survival in LHF-PH ($P < 0.05$). When adjusted for age, sex and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels using multivariable cox regressions, only WIF-1 remained prognostic [hazard ratio (95% confidence interval)] [1.013 (1.001–1.024)]. WIF-1 levels in LHF-PH patients also correlated with the mean right atrial pressure ($r_s = 0.42$; $P < 0.01$), stroke volume index ($r_s = 0.41$; $P < 0.01$), cardiac index ($r_s = -0.42$; $P < 0.01$), left ventricular stroke work index ($r_s = -0.41$; $P < 0.01$), and NT-proBNP ($r_s = 0.63$; $P < 0.01$).

Conclusions The present study demonstrated that LHF-PH patients have higher plasma WIF-1 levels than healthy controls, suggesting that plasma WIF-1 may be a potential future prognostic biomarker in LHF-PH. Its prognostic capability could be further refined by including it in a multi-marker panel. Further studies are needed to establish the potential role of WIF-1 in LHF-PH pathophysiology in larger cohorts to determine its clinical applicability.

Keywords Biomarkers; Coagulation proteins; WIF1; Haemodynamics; PD-L2; TFPI-2

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**Correspondence to: Kriss Kania, Department of Cardiology, Skåne University Hospital, 221 85 Lund, Sweden. Email: kriss.kania1@gmail.com*

Introduction

Heart failure (HF) is a prevalent condition that is present in roughly 1–2% of the adult population and in over 10% in people aged >70 years in developed countries.[1] The most common aetiology of pulmonary hypertension (PH) is left heart disease, predominantly due to left HF (LHF), accounting for approximately 65–80% of all PH cases.[2] PH is a common complicating condition in LHF associated with an increased morbidity and worse outcome. Although the complete pathophysiology of PH due to LHF (LHF-PH) is yet to be fully understood, LHF-PH stems from a backward propagation of increased left-sided filling pressures, occurring as a consequence of systolic and/or diastolic dysfunction in the left ventricle.[2,3] This may subsequently trigger increased endothelin expression, pulmonary vasoconstriction, and pulmonary vascular

remodelling, further progressing the PH and increasing the burden on the right ventricle, ultimately leading to biventricular failure and death.[4–6]

The use of proteomics has been proposed to aid in the understanding of the pathophysiology, diagnostics, risk stratification, and prognostication in HF.[6,7] Although BNP/NT-proBNP are the only widely used biomarkers with diagnostic and prognostic potential,[1] the increasing incidence and the multifaceted pathophysiology of HF call for further prognostic refinement. To improve the prognostic potential of biomarkers in HF, and to better individualize the care of each patient, a multi-marker approach reflecting each pathophysiological pathway has been proposed to be of potential value.[7]

HF is associated with abnormalities in several molecular pathways, one of them being the coagulation system. A hypercoagulable state can be observed in HF, leading to haemostatic complications due to increased thrombogenesis. In relation to this, changes can be observed in the concentration of coagulation related proteins, such as markers of haemostasis, platelet activity, endothelial dysfunction, and fibrinolysis.[8–10] Wnt signalling proteins, commonly associated with the formation of several organ systems, have recently been implicated in cardiac pathophysiology including HF.[11] HF is also associated with both local and systemic activation of inflammation. Elevated levels of pro-inflammatory cytokines in HF can lead to the degradation of the extracellular matrix by activating matrix metalloproteinases.[12] The activated inflammatory pathways also contribute to coagulatory abnormalities.[8–10] Proteins involved in these and other pathophysiological pathways could provide further clues to the pathophysiology of HF and LHF-PH and/or reflect its progression. Moreover, they could be used clinically to aid in diagnosis, risk stratification, and prognostic estimations and thus potentially be used as biomarkers in LHF-PH. Therefore, we aimed to investigate the prognostic value of 33 proteins corresponding to the previously mentioned pathways, in patients with LHF-PH.

Materials and methods

Study population and demographic data

The participants comprised 20 healthy controls without a past medical history of myocardial infarction, HF, diabetes mellitus, or atrial fibrillation, as well as 67 patients with LHF-PH diagnosed at Skåne's University Hospital in Lund, Sweden. Of the 67 LHF-PH patients, 20 had undergone heart transplantation (HT) at the time of blood sample collection. All HT patients had advanced HF and were individually treated with maximal tolerated doses of heart failure treatment. The heart transplanted patients were followed up 1 year after HT, thus comprising two subgroups, that is, before (pre-HT) and after HT (post-HT). A left ventricular assist device was implanted in eight of the 20 HT patients as a bridge to transplantation. One patient exhibited PH after HT, and was therefore excluded from the HT groups, but was still included in the LHF-PH group. Five patients were excluded due to haemodynamically unconfirmed PH diagnosis.

All participants were ≥18 years old and provided written, informed consent. The study was conducted in accordance with the ethical standards defined in the declarations of Helsinki and Istanbul and was approved by the ethical board in Lund, Sweden (Dnr: 2010/114, 2010/442, 2011/368, 2011/777, 2014/92, 2015/270).

Blood samples

The present study was based on data from blood samples collected between October 2011 and February 2017 for the Lund Cardio Pulmonary Registry (LCPR), stored at −80°C. LCPR is a cohort of Region Skåne's biobank, initiated by Göran Rådegran in September 2011, and includes both blood samples and clinical data. Venous blood plasma samples were collected from LHF-PH patients during right heart catheterization (RHC) and additionally at the routine 1-year follow-up from the HT recipients. Venous blood samples were also collected from healthy controls. The participants were not fasting at the time of blood sampling.

Analysis of plasma proteins

Proximity extension assay (PEA) was used to analyse 33 plasma proteins and N-terminal pro-brain natriuretic peptide (NT-proBNP) using Proseek Multiplex immunoassay kits (Cardiovascular disease II, III, and Oncology II panels, Olink Proteomics, Uppsala, Sweden). PEA operates by mixing a fixed plasma sample volume with oligonucleotide-labelled antibodies, called probes. These probes then bind pairwise to the target protein and create a polymerase chain reaction target sequence in a polymerization event. This sequence can then be detected and quantified using quantitative real-time polymerase chain reaction.[13] The plasma proteins included were a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13), bone morphogenetic protein 6 (BMP-6), delta-like protein 1 (DLL-1), dickkopf-related protein 1 (Dkk-1), follistatin, growth hormone, growth/differentiation factor 2 (GDF-2), heat shock 27 kDa protein (HSP 27), haem oxygenase 1 (HO-1), mothers against decapentaplegic homolog 5 (MAD homolog 5), neurogenic locus notch homolog protein 3 (Notch 3), NF-kappa-B essential modulator (NEMO), plasminogen activator inhibitor 1 (PAI-1), poly [ADP-ribose] polymerase 1 (PARP-1), programmed cell death 1 ligand 2 (PD-L2), protein delta homolog 1 (DLK-1), proteinase-

activated receptor 1 (PAR-1), R-spondin-3, serine/threonine-protein kinase 4 (STK-4), spondin-1, spondin-2, superoxidase dismutase [Mn], mitochondrial (SOD2), TGF-beta receptor type-2 (TGFR-2), thrombomodulin, thrombopoietin, tissue factor, tissue factor pathway inhibitor (TFPI), (TFPI-2), tissue-type plasminogen activator 2 (tPA), urokinase plasminogen activator surface receptor (uPAR), urokinase-type plasminogen activator (uPA), von Willebrand factor (vWF), and Wnt inhibitory factor 1 (WIF-1).

To control the quality of the data, protein level normalization and adjustments for inter-plate variations were achieved by adding internal controls to each sample and adding external controls as separate samples, respectively. The level of each protein analysed using PEA is expressed using arbitrary units (AU) on a linear, normalized protein expression scale.[13]

Assessing haemodynamics and renal function

Haemodynamic measurements of the LHF-PH and post-HT groups were taken during routine clinical examinations of HF patients evaluated for a de novo heart transplant and at the 1-year follow-up after HT, respectively. Haemodynamic measurements were acquired via RHC using a Swan Ganz catheter (Baxter Healthcare Corp, Santa Ana, CA) inserted principally via the right internal jugular vein in supine position. LHF-PH was diagnosed by experienced cardiologists and defined according to the prevailing ESC/ERS guidelines as a mean pulmonary arterial pressure (mPAP) $\geq$ 25 mmHg combined with a pulmonary arterial wedge pressure (PAWP) $>$ 15 mmHg.[4] Isolated post-capillary PH was defined as a pulmonary vascular resistance (PVR) $\leq$ 3 WU (wood units) and/or a diastolic pressure gradient (DPG) $<$ 7 mmHg. Combined post-capillary and pre-capillary PH was defined as PVR $>$ 3 WU and/or DPG $\geq$ 7 mmHg.[4] Non-invasive mean arterial pressure (mAP), mPAP, mean right atrial pressure (mRAP), PAWP, systolic pulmonary arterial pressure (sPAP), diastolic pulmonary arterial pressure (dPAP), arterial oxygen saturation (SaO_2), and mixed venous oxygen saturation (SvO_2) were measured during RHC. Cardiac output (CO) was measured using thermodilution.

Body surface area (BSA) was calculated using the DuBois method (BSA = $0.007184 \times \text{Height}^{0.725} \times \text{Weight}^{0.425}$).[14] Additional haemodynamic parameters were calculated using the following formulas: cardiac index (CI) = CO/BSA, stroke volume index (SVI) = CI/heart rate (HR), PVR = transpulmonary pressure gradient (TPG)/CO, pulmonary vascular resistance index (PVRI) = PVR/BSA, pulmonary arterial compliance (PAC) = SV/(sPAP-dPAP), TPG = mPAP-PAWP, left ventricular stroke work index (LVSWI) = (mAP $-$ PAWP) $\times$ SVI, and right ventricular stroke work index (RVSWI) = (mPAP $-$ mRAP) $\times$ SVI.

The revised Lund–Malmö formula was used to calculate a creatinine-based estimate of the glomerular filtration rate (eGFR) at the time of RHC.[15] Arteriovenous oxygen difference was calculated using SaO_2 and SvO_2. HF and immunosuppression medications were used according to the prevailing consensus statement of ESC and the International Society for Heart and Lung Transplantation.[1,16]

Statistical analysis and study setup

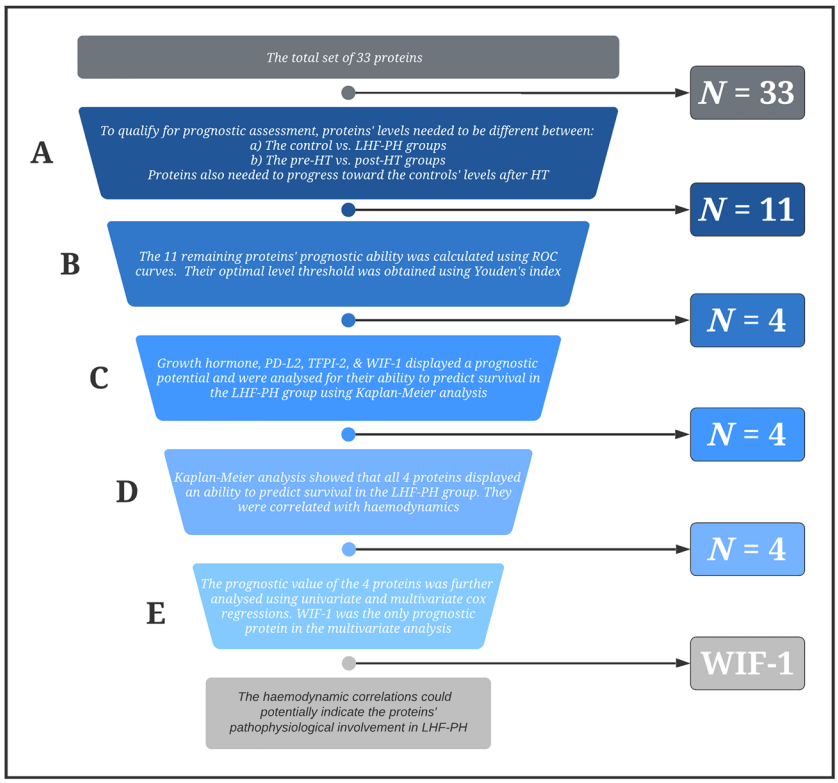
Continuous descriptive variables are presented as medians and interquartile ranges. Data were tested visually using histograms for normal distribution prior to statistical analysis. Plasma proteins whose levels displayed a difference in the pre-HT vs. post-HT groups, controls vs. LHF-PH groups, as well as a normalization pattern towards the levels of the controls after HT (*Figure 1A*), qualified for prognostic analysis. Wilcoxon signed-rank test and Mann–Whitney *U* test were applied to measure the difference in the proteins' levels between the groups and were used for paired and unpaired data, respectively. Subsequently, receiver operating characteristics (ROC) curves were plotted from the LHF-PH group's levels of the remaining proteins using the Wilson/Brown method. The optimal protein level thresholds were determined using Youden's index (*Figure 1B*). Next, using the optimal protein threshold, survival analysis was performed using Kaplan–Meier analysis. Events were defined as transplantation-free survival. The difference in survival between the two groups was measured using the logrank/Mantel–Cox test (*Figure 1C*). The levels of proteins that displayed a difference in survival in the Kaplan–Meier analyses were later correlated with haemodynamic parameters in the LHF-PH group using Spearman's rank coefficient (r_s) (*Figure 1D*). The prognostic ability of the proteins was also assessed using univariable and multivariable cox regression models. The multivariable models were adjusted for sex, age, and levels of NT-proBNP (*Figure 1E*). To account for mass significance, the false discovery rate (FDR) was calculated using the two-stage step-up method of Benjamini, Krieger, and Yekutieli, applying a *Q* value of 1% for plasma proteins and for correlations between plasma proteins and haemodynamic parameters. *P* values less than 0.05 were deemed statistically significant in the ROC, Kaplan–Meier and Cox regression analyses. Remaining *P* values were deemed statistically significant if they were less than the attained FDR thresholds. All analyses were performed using GraphPad Prism version 9.1.2 (GraphPad Software, La Jolla, CA, USA).

Results

Population characteristics

The population characteristics have in part previously been described[17,18] and can be found in *Tables 1* and *S1*. The me-

Figure 1 Summary of the study setup. Statistical significance in (A) was based on $P < 0.01$ using a false discovery rate of <1%. Statistical significance in (D) was based on $P < 0.004$ using a false discovery rate, Q value at 1%. HT, heart transplantation; LHF-PH, left heart failure with pulmonary hypertension; PD-L2, programmed cell death 1 ligand 2; ROC, receiver operating characteristics; TFPI-2, tissue factor pathway inhibitor 2; WIF-1, Wnt inhibitory factor 1.



dian follow-up of the patients was 6.2 years with a range of 3.6–8.8 years, with either transplantation or death as events. The total amount of events in the LHF-PH group was 53, constituted of 36 patients who received a heart transplant and 17 who died without transplantation. A cut-off time of 73 months for the follow-up time of WIF-1 was applied. Patients were censored on 21 August 2020.

Difference in proteins' levels between study population groups

Out of the total 33 proteins, 11 proteins had a difference between the control and LHF-PH groups, and between the pre-HT and post-HT groups ($P < 0.01$; FDR < 1%), and whose levels after HT progressed towards the controls (*Table S2*). These proteins included BMP-6, follistatin, growth hormone, MAD Homolog 5, Notch 3, PD-L2, Spondin-1, Spondin-2, TFPI-2, U-PAR, and WIF-1. The remaining proteins did not progress to prognostic evaluation.

Growth hormone, PD-L2, TFPI-2, and WIF-1 predicted transplantation-free survival in patients with LHF-PH

The 11 remaining proteins were analysed using ROC curves to determine the optimal protein level threshold for survival analysis (*Table S3*). Of the remaining 11 proteins, four had statistically significant areas under the ROC curve (AUC) ($P < 0.05$). These included growth hormone (AUC = 0.73; $P = 0.0090$), PD-L2 (AUC = 0.67; P = 0.0493), TFPI-2 (AUC = 0.77; $P = 0.0020$), and WIF-1 (AUC = 0.83; $P = 0.0002$). The remaining seven proteins did therefore not proceed to further analysis.

Survival calculations using the thresholds obtained from ROC analyses showed that LHF-PH patients with high levels of growth hormone ($P = 0.0009$; *Figure 2B*), PD-L2 ($P = 0.0103$; *Figure 2F*), TFPI-2 ($P = 0.0011$; *Figure 2D*), and WIF-1 ($P < 0.0001$; *Figure 3B*) had significantly lower transplantation-free survival compared with those with low plasma levels.

Table 1 Demographics of the study population

	Controls (n = 20)		LHF-PH (n = 67)[a]		Pre-HT (n = 19)		Post-HT (n = 19)	
	n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)
Female, n (%)	10 (50)		28 (42)		3 (16)		3 (16)	
Age (years)	20	41 (27–51)	67	63 (51–75)	19	52 (49–64)	19	52 (49–64)
Height (cm)	20	176 (166–181)	67	174 (164–180)	19	175 (170–181)	19	175 (170–181)
Weight (kg)	19	73 (67.9–79.3)	67	80 (70.4–90.1)	19	77 (68.5–90)	19	77 (68.5–90)
mAP (mmHg)	20	95 (88.8–99.8)	67	89 (79–99)	19	82 (78–90)	19	101 (90–106)
SaO_2 (%)	20	98 (97–98)	67	94.8 (91.8–96.4)	19	95.9 (93–96.4)	17	97 (96–98)
BSA (m^2)	19	1.9 (1.8–2.0)	67	1.9 (1.8–2.1)	19	2 (1.8–2)	19	2 (1.8–2)
Creatinine (μmol/L)			63	108 (86–136)	18	110 (89.5–125)	19	104 (97–121)
eGFR (mL/min/1.73 m^2)			63	53.9 (39.6–66.2)	18	62.8 (54.1–70.5)	19	71 (46.2–72.7)
NT-proBNP (AU)	20	1.1 (1.1–1.2)	67	13 (7.6–32.1)	19	28.4 (17.1–44.6)	19	2 (1.5–6.5)
Atrial fibrillation, n (%)			37 (55)		7 (37)		-	
Hypertension, n (%)			27 (40)		2 (11)		1 (5)	
Diabetes mellitus, n (%)			14 (21)		1 (5)		6 (32)	
HFrEF (EF $<$ 50%)			36 (54)		18 (95)		-	
HFpEF (EF $\geq$ 50%)			31 (46)		1 (5)		-	
Ipc-PH			30 (45)		10 (53)[b]		-	
Cpc-PH			37 (55)		9 (47)		-	
β-Blockers			59 (88)		18 (95)		3 (16)	
ACEi			30 (45)		9 (47)		-	
MRA			28 (42)		8 (42)		2 (11)	
Furosemide			40 (60)		18 (95)		7 (37)	
Prednisolone			-		-		18 (95)	
Cyclosporine			1 (2)		-		2 (11)	
Tacrolimus			-		-		17 (90)	
Mycophenolate mofetil			-		-		15 (79)	
Azathioprine			1 (2)		-		3 (16)	

–, data not available; ACEi, angiotensin-converting enzyme inhibitor; AU, arbitrary units; BSA, body surface area; Cpc-PH, combined post-capillary and pre-capillary pulmonary hypertension; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; Ipc-PH, isolated post-capillary pulmonary hypertension; IQR, interquartile range; LHF-PH, pulmonary hypertension due to left heart failure; mAP, mean arterial pressure; MRA, mineralocorticoid receptor antagonist; SaO_2, arterial oxygen saturation.
[a]A borderline diagnostic PAWP of 15 mmHg was found in five patients. They underwent either a saline fluid overload or leg lifting and/or a multidisciplinary discussion to determine the diagnosis.
[b]Severe orthopnoea prevented a successful assessment of pulmonary arterial wedge pressure (PAWP) in one patient. A subsequent right heart catheterization performed after treatment with furosemide and levosimendan showed the patient had Ipc-PH.

The prognostic proteins correlated with several haemodynamic parameters

Correlations between protein levels in the LHF-PH group with haemodynamic parameters and NT-proBNP were performed to assess the potential relationship to LHF-PH (*Table S4*). Growth hormone, PD-L2, TFPI-2, and WIF-1 correlated with several haemodynamic parameters, as well as NT-proBNP. Growth hormone, TFPI-2 and WIF-1 correlated with CI, LVSWI, mRAP, NT-proBNP, and SVI ($P < 0.004$). PD-L2 correlated with mPAP, mRAP, NT-proBNP, and PVR ($P < 0.004$).

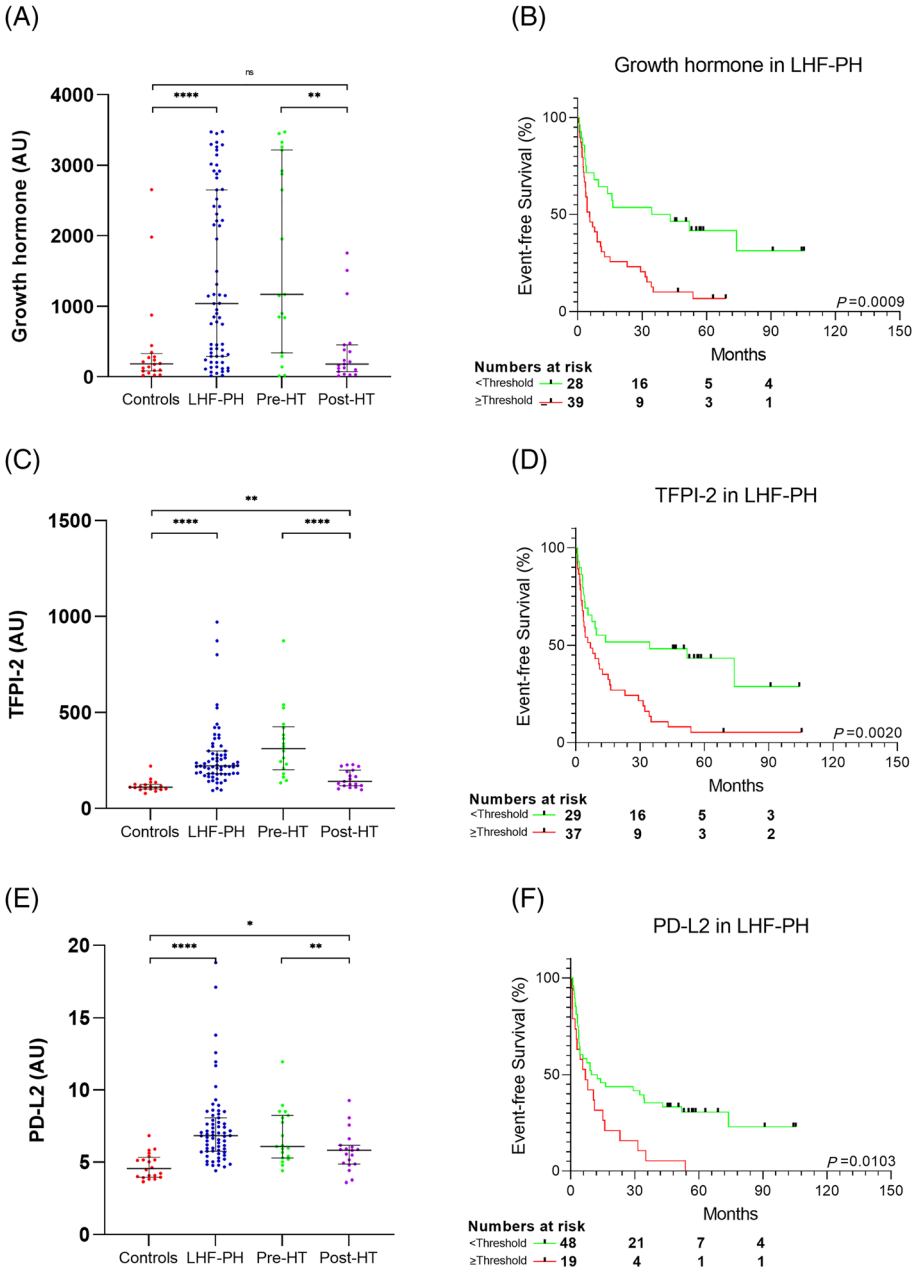
WIF-1 remained prognostic after adjusting for age, sex, and NT-proBNP

The prognostic ability of the proteins was further analysed using cox regression models (*Table 2*). Univariable Cox regression analysis displayed worse transplantation-free survival in relation to an increase in the levels of growth hormone (HR 1.000; $P = 0.0095$), TFPI-2 (HR 1.002; $P = 0.0306$), and WIF-1 (HR 1.016; $P = 0.0004$) in the LHF-PH patient group. Age (HR 0.973; $P = 0.0011$), male sex (HR 2.132; $P = 0.0114$), and NT-proBNP (HR 1.019; $P = 0.0002$) were also significant predictors in the univariable model. When the proteins were adjusted for age, sex, and NT-proBNP in the multivariable models, only WIF-1 (HR 1.013; $P = 0.0290$) displayed an ability to predict the transplantation-free survival of the LHF-PH group.

Discussion

Biomarkers, including the use of multi-marker panels, have been proposed as a means of refining risk stratification and prognostic estimations in patients with HF,[6] and therefore potentially also LHF-PH. Such a panel would ideally include biomarkers that reflect every pathophysiological pathway, as a panel consisting of markers strongly correlated with each other does not add much in terms of predictive value.[7,19] In the present study, we found that the plasma level of growth hormone, PD-L2, TFPI-2, and WIF-1 were elevated in LHF-PH patients and decreased towards the levels of the controls after HT. In a Kaplan–Meier analysis, these proteins were also able to prognosticate transplantation-free survival in LHF-PH

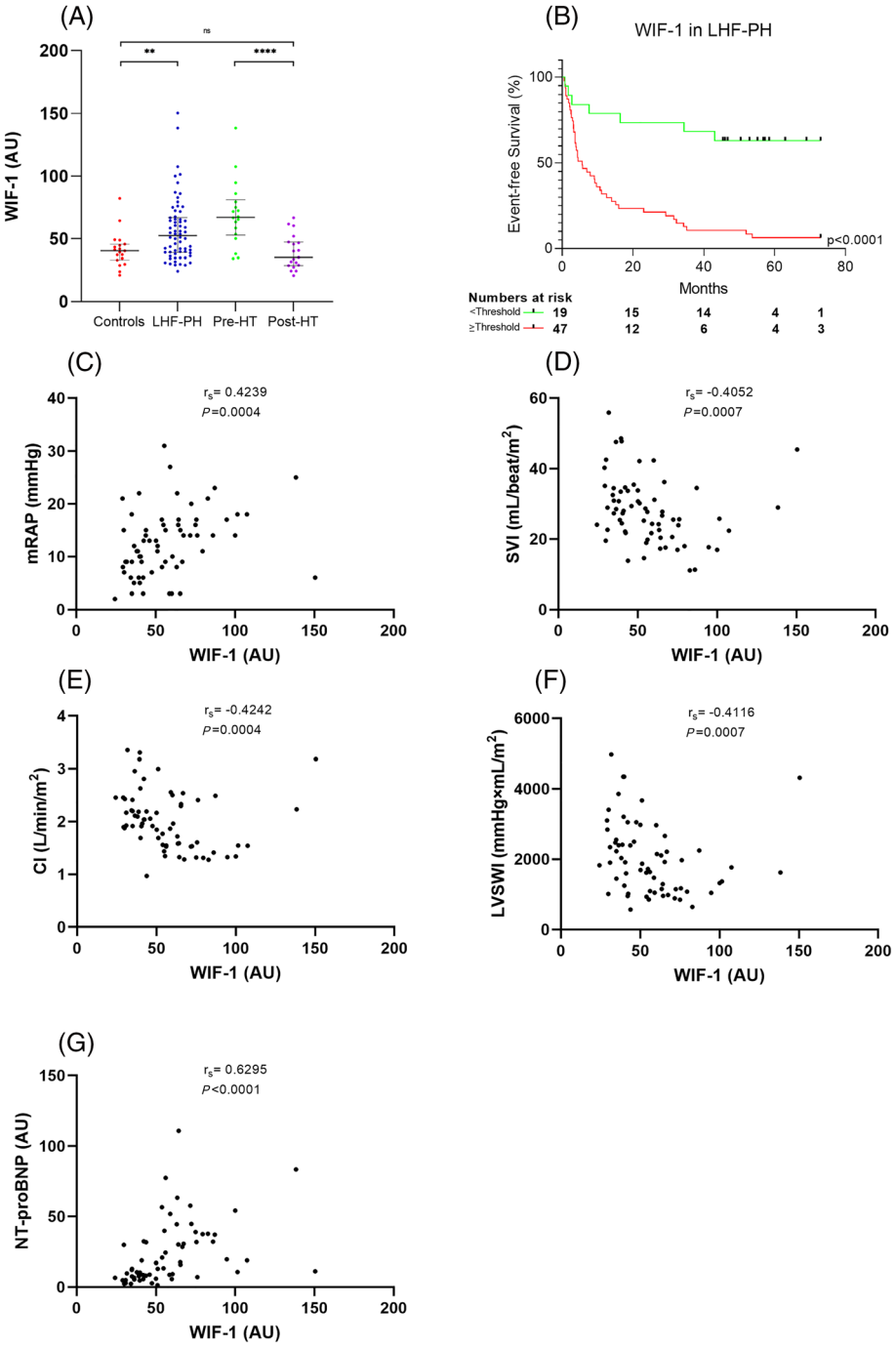
Figure 2 (A, C, and E) Plasma levels of growth hormone, programmed cell death 1 ligand 2 (PD-L2), and tissue factor pathway inhibitor 2 (TFPI-2) in controls, patients with pulmonary hypertension due to left heart failure (LHF-PH), and in patients before (pre-HT) and after (post-HT) heart transplantation. (B, D, and F) Kaplan–Meier analysis and numbers at risk of LHF-PH patients with growth hormone, PD-L2, and TFPI-2 levels either above or below the threshold calculated using ROC analysis. AU indicates arbitrary units; *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$; ns, not significant.



patients and may therefore be potential future biomarker candidates. When adjusted for age, sex, and NT-proBNP in the multivariable Cox regression models, only WIF-1 retained its prognostic ability. Additionally, plasma WIF-1 levels correlated with several haemodynamic and cardiac parameters, further indicating its association with cardiac function.

WIF-1 is a secreted protein that binds and inhibits Wnt proteins[20] and comprises one of several endogenous Wnt antagonists.[11] The Wnt protein family includes 19 secreted glycoproteins and acts through three Wnt signalling cascades that are involved in a multitude of cellular processes, including cell proliferation, differentiation, and migration. They have important roles in the embryological development of many organ systems,[11] including cardiogenesis[11,21,22] and lung development.[23] Although Wnt signalling is largely inactive in the healthy adult heart, recent studies have found that

Figure 3 (A) Plasma levels of Wnt inhibitory factor 1 (WIF-1) in controls, in patients with pulmonary hypertension due to left heart failure (LHF-PH), and in patients before (pre-HT) and after (post-HT) heart transplantation. (B) Kaplan–Meier analysis and numbers at risk of LHF-PH patients with WIF-1 levels either above or below the threshold calculated using ROC analysis. (C–G) Correlations between the level of WIF-1 and haemodynamic parameters. AU indicates arbitrary units; CI, cardiac index; LVSWI, left ventricular stroke work index; mRAP, mean right atrial pressure; ns, not significant; NT-proBNP, N-terminal pro-brain natriuretic peptide; SVI, stroke volume index; $^{**}P < 0.01$; $^{****}P < 0.0001$.



Wnt signalling can be reactivated in the adult heart in certain pathological states.[11,24]

Two of the main causes of HF are pathological cardiac hypertrophy and myocardial infarction (MI).[24] Wnt signalling has been implicated in both, and has been reported to influence their progression into HF.[11] Malekar *et al.*[25] reported that Wnt signalling is reactivated in the myocardium during a state of prolonged pressure overload, such as in a chronic

Table 2 Univariable and multivariable Cox regression analyses of growth hormone, PD-L2, TFPI-2, and WIF-1

Univariable Cox regression		HR (95% CI)	*P* value
Growth hormone (AU)		1.000 (1.000–1.001)	0.0095*
PD-L2 (AU)		1.059 (0.968–1.158)	0.2110
TFPI-2 (AU)		1.002 (1.000–1.003)	0.0306*
WIF-1 (AU)		1.016 (1.007–1.025)	0.0004*
Age (years)		0.973 (0.957–0.989)	0.0011*
NT-proBNP (AU)		1.019 (1.009–1.029)	0.0002*
Sex (M)		2.132 (1.186–3.832)	0.0114*
Multivariable Cox regression			
Model 1	Growth hormone (AU)	1.000 (1.000–1.000)	0.3571
	Age (years)	0.983 (0.964–1.002)	0.0805
	NT-proBNP (AU)	1.012 (1.000–1.025)	0.0512
	Sex (M)	1.324 (0.671–2.615)	0.4184
Model 2	PD-L2 (AU)	1.022 (0.922–1.132)	0.6843
	Age (years)	0.979 (0.960–0.999)	0.0389*
	NT-proBNP (AU)	1.012 (0.999–1.025)	0.0796
	Sex (M)	1.399 (0.715–2.735)	0.3266
Model 3	TFPI-2 (AU)	0.999 (0.997–1.001)	0.3170
	Age (years)	0.979 (0.960–0.998)	0.0317*
	NT-proBNP (AU)	1.018 (1.002–1.033)	0.0234*
	Sex (M)	1.535 (0.781–3.016)	0.2135
Model 4	WIF-1 (AU)	1.013 (1.001–1.024)	0.0290*
	Age (years)	0.980 (0.962–0.999)	0.0366*
	NT-proBNP (AU)	1.007 (0.993–1.021)	0.3323
	Sex (M)	1.436 (0.719–2.867)	0.3052

AU, arbitrary units; CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; PD-L2, programmed cell death 1 ligand 2; TFPI-2, tissue factor pathway inhibitor 2; WIF-1, Wnt inhibitory factor 1.
The multivariable models were adjusted for age, sex, and NT-proBNP.
*$P < 0.05$.

afterload increase seen in aortic stenosis. Furthermore, they found that Wnt signalling pathways have a role in maladaptive myocardial remodelling and dysfunction and are critical in the development of pathological cardiac hypertrophy. Several studies have shown Wnt signalling to be present in myofibroblasts of granulation tissue that replaces dead cardiomyocytes post-MI.[26,27] Myofibroblast migration and differentiation has also been shown to be affected by Wnt signalling.[28] It has thus been speculated that Wnt inhibition may have a positive effect on ventricular remodelling post-MI.[24]

WIF-1 has also been associated with cardiovascular disease and various forms of PH. One study found that elevated circulating WIF-1 levels were associated with an increased risk of future cardiovascular disease and that it was a significant predictor of future cardiovascular disease. They speculated that WIF-1 might have a protective, anti-atherosclerotic effect.[29] Askevold *et al*.[30] found in a study that WIF-1 levels were elevated in patients with symptomatic atherosclerosis and that WIF-1 successfully predicted mortality in patients with atherosclerosis. Furthermore, concordant with our findings, they found that WIF-1 levels were correlated with the degree of myocardial function and the levels of NT-proBNP. A recent study showed that WIF-1 levels were elevated in precapillary PH compared with controls. WIF-1 correlated with various haemodynamic and cardiac parameters. High WIF-1 levels were also associated with a worse prognosis in precapillary PH.[31] These results, while analysing precapillary PH and not LHF-PH, are similar to our own findings and further illuminate WIF-1 to be a potential future biomarker in PH.

Wnt signalling has also been found to be involved in the regulation of smooth muscle cell proliferation in the lung of patients with pulmonary arterial hypertension (PAH; group 1 PH), which is a hallmark of the disease and leads to narrowing of the vessels and disease progression.[32] Several Wnt proteins are also expressed in endothelial cells, and there is increasing evidence that Wnt signalling could play an important role in regulating vascular remodelling during both normal and pathological angiogenesis.[33] Because vascular remodelling may play a part in the progression of all PH groups,[34] it could implicate Wnt signalling being part of the pathophysiology and progression of PH. Although the pathophysiology varies between the different forms of PH, these findings show that there is evidence for WIF-1 being related to the development of PH, making it a very interesting marker for further studies.

Strengths and limitations

The invasive haemodynamic assessments and the use of PEA for measuring the levels of the plasma proteins are considered strengths of the present study, the latter due to its excellent sensitivity and specificity.13 Even though absolute concentrations are not obtained using PEA, it is not consid-

ered a limitation, as the aim of the study did not require absolute protein concentrations due to the study's explorative nature. Confounding factors such as medication intake and other comorbidities may have impacted the results of the present study. Although WIF-1 levels correlated with several haemodynamic parameters, thus partially allowing to avoid the limitation of post-HT medication, co-morbidities are still a potential confounder. To our knowledge, the effect of many immunosuppressive medications on Wnt signalling in humans remains largely unknown. However, animal and *in vitro* studies have suggested cyclosporine A, mycophenolate, and prednisolone as potential upregulators of the Wnt pathway.[35–37] Moreover, the results do not imply causality, and we are not able to draw any mechanistical conclusions related to the function of the proteins. Furthermore, the relatively low number of study participants has posed limitations on the ability to correct for confounding variables. Additionally, echocardiograms of the control group were not performed to exclude the possibility of pulmonary hypertension. This risk was mitigated by carefully choosing controls without any clinical symptoms of heart failure or pulmonary hypertension. Nevertheless, this study illuminates WIF-1 as a potential novel biomarker candidate in LHF-PH.

Conclusions

The Wnt signalling pathway, including WIF-1, has been implicated in a variety of pathways that contribute to the pathophysiology and progression of both HF and PH. The present study shows that WIF-1 is elevated in LHF-PH patients compared with controls. Also, LHF-PH patients with elevated WIF-1 levels had a significantly worse prognosis, which remained unchanged when adjusted for age, sex, and NT-proBNP levels. Our results suggest that WIF-1 levels are associated with LHF-PH and may aid in determining its prognosis. Taken together, our study demonstrates that WIF-1 may be an interesting future prognostic biomarker candidate. Additional studies are needed, with a mechanistic emphasis to define the role of WIF-1 in the pathophysiology of PH and in larger cohorts to better evaluate its prognostic ability.

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Conflict of interest

Mr Kriss Kania reports no conflicts of interest. Dr Göran Rådegran reports unrestricted research grants from ALF and Janssen during the conduct of the study.

Drs Abdulla Ahmed and Salaheldin Ahmed report personal lecture fees from Janssen outside the submitted work. Dr Göran Rådegran reports personal lecture fees from Actelion Pharmaceuticals Sweden AB, GlaxoSmithKline, Bayer Healthcare, Janssen, and Nordic Infucare outside the submitted work.

Dr Göran Rådegran is and has been primary, or co-investigator, investigator in clinical PAH trials for Acceleron, Actelion Pharmaceuticals Sweden AB, GlaxoSmithKline, Pfizer, Bayer, Janssen, and United Therapeutics and in clinical heart transplantation immunosuppression trials for Novartis. The companies had no role in the data collection, analysis, and interpretation and had no right in disapproving of the manuscript.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Patients' haemodynamic parameters, including before, and at the one-year follow-up after HT.
Table S2. Patients' protein levels.
Table S3. Receiver operating characteristics (ROC) analysis of protein levels in the LHF-PH group.
Table S4. Correlations between protein levels in the LHF-PH group with haemodynamic parameters and NT-proBNP.

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