

Supplementary tables and figures

Clinical phenotyping of plasma thrombospondin-2 reveals relation to right ventricular structure and function in pulmonary hypertension

Anna M. Dittrich, Julia Mienert, MSc, Julian Pott, MD, Lena Engels, PhD, Christoph Sinning, MD, Jan K. Hennigs, MD, Hans Klose, MD, Lars Harbaum, MD

Supplementary Table 1: Time between clinical tests and sampling in patients with pulmonary hypertension (PH) or disease controls (DC).

Clinical test	Percentile/quartile of time in days and missing clinical tests						
	10%	25%	Median	75%	90%	Missing, N	Missing, %
WHO-FC	0	0	0	0	4	0	0
6MWD	0	0	0	6	84	71	33
PFT	0	0	0	9	36	9	4
TTE	0	0	7	86	155	13	6
RHC	0	1	147	761	1525	0	0
Lab	0	0	0	2	6	1	0

Supplementary Table 2: Results from linear regression models associating clinical measures with TSP2 levels in patients with pulmonary hypertension (PH), independent of age at sampling, body mass index and gender. Bonferroni procedure was applied to obtain q-values in the primary analysis.

Clinical variable	In patient with PH (primary analysis)			
	Estimate	SE	p-value	q-value
GGT, UI/l	0.13	0.02	2.18E-08	3.38E-06
MRAP, mmHg	1.41	0.25	6.47E-08	1.00E-05
NT-proBNP, 100 ng/l	0.00	0.00	4.13E-07	6.40E-05
RAA, cm ²	0.61	0.14	1.30E-05	2.02E-03
RVFAC, %	-0.54	0.12	1.86E-05	2.88E-03
TAPSE, mm	-1.06	0.26	5.59E-05	8.67E-03
eGFR, ml/min	-0.21	0.05	6.57E-05	1.02E-02
RVEDP, mmHg	0.89	0.22	6.86E-05	1.06E-02
TAPSE/SPAP, 0.01 mm/mmHg	-0.38	0.09	8.88E-05	1.38E-02
SVI, ml/m ²	-0.42	0.11	1.59E-04	2.47E-02
SvO ₂ , %	-0.53	0.14	1.80E-04	2.80E-02
SV, ml	-0.21	0.05	1.99E-04	3.08E-02
MPAP/MBP, 0.1 arbitrary unit	2.35	0.67	5.85E-04	9.07E-02
LVEDA, cm ²	-0.77	0.22	7.54E-04	1.17E-01
PAC, ml/mmHg	-3.46	1.01	8.10E-04	1.25E-01
RVESA, cm ²	0.70	0.20	8.17E-04	1.27E-01
DPAP, mmHg	0.36	0.11	8.47E-04	1.31E-01
MPAP, mmHg	0.28	0.08	8.50E-04	1.32E-01
WHO-FC, class	5.16	1.55	1.07E-03	1.65E-01
PVR, WU	0.96	0.29	1.14E-03	1.77E-01
RVESP, mmHg	0.17	0.05	1.64E-03	2.54E-01
INR, arbitrary unit	5.56	1.74	1.66E-03	2.58E-01
AF, present	9.13	2.87	1.70E-03	2.63E-01
6MWD, % pred	-0.16	0.05	1.78E-03	2.75E-01
RVEDD basal, mm	0.45	0.14	1.86E-03	2.89E-01
CO, l/min	-2.64	0.88	3.11E-03	4.82E-01
SPAP, mmHg	0.16	0.05	3.88E-03	6.01E-01
B01AB Heparin group, present	26.31	9.12	4.40E-03	6.82E-01
TLC, % pred	-0.19	0.07	5.77E-03	8.95E-01
TPG, mmHg	0.23	0.09	9.00E-03	1.00E+00
CI, l/min/m ²	-4.06	1.54	9.04E-03	1.00E+00
DPD, mmHg	0.30	0.12	9.13E-03	1.00E+00
C07A BETA BLOCKING AGENTS, present	6.85	2.60	9.20E-03	1.00E+00
FVC, % pred	-0.17	0.06	1.07E-02	1.00E+00
A06A DRUGS OR CONSTIPATION, present	22.99	9.20	1.34E-02	1.00E+00
Peripheral edema, present	7.32	2.93	1.37E-02	1.00E+00
TRV, m/s	4.50	1.82	1.49E-02	1.00E+00
HR, bpm	0.22	0.09	1.56E-02	1.00E+00
6MWD, m	-0.02	0.01	1.87E-02	1.00E+00

C01 CARDIAC THERAPY, present	16.92	7.19	1.96E-02	1.00E+00
Fibrinogen, g/l	5.19	2.25	2.27E-02	1.00E+00
LVESA, cm ²	-0.66	0.30	2.88E-02	1.00E+00
AST, UI/l	0.18	0.08	3.43E-02	1.00E+00
CRP, mg/l	0.56	0.26	3.50E-02	1.00E+00
mPAWP, mmHg	0.47	0.22	3.54E-02	1.00E+00
Weight, kg	0.34	0.16	3.72E-02	1.00E+00
FEV1, % pred	-0.12	0.06	4.10E-02	1.00E+00
TLC, l	-2.12	1.08	5.20E-02	1.00E+00
N02A OPIOIDS, present	9.05	4.97	7.02E-02	1.00E+00
C03 DIURETICS, present	4.50	2.51	7.47E-02	1.00E+00
Pleural effusion, present	7.23	4.12	8.06E-02	1.00E+00
RVEDA, cm ²	0.32	0.19	8.92E-02	1.00E+00
IVPWd, mm	0.92	0.54	8.99E-02	1.00E+00
Mitral valve disease, present	4.16	2.46	9.20E-02	1.00E+00
N03A ANTIPILEPTICS, present	6.67	3.94	9.22E-02	1.00E+00
HCT, %	-0.29	0.17	9.38E-02	1.00E+00
Pulse pressure, mmHg	0.13	0.08	9.57E-02	1.00E+00
ITGV, % pred	-0.07	0.05	1.06E-01	1.00E+00
History of syncope, present	-4.93	3.16	1.21E-01	1.00E+00
E/e', arbitrary unit	0.41	0.26	1.24E-01	1.00E+00
ALT, UI/l	0.11	0.07	1.41E-01	1.00E+00
H02A CORTICOSTEROIDS FOR SYSTEMIC USE, present	-7.21	4.92	1.44E-01	1.00E+00
R03D OTHER SYSTEMIC DRUGS FOR OBSTRUCTIVE AIRWAY DISEASES, present	-10.55	7.20	1.44E-01	1.00E+00
Aortic valve disease, present	4.00	2.76	1.49E-01	1.00E+00
FVC, l	-2.31	1.59	1.49E-01	1.00E+00
L04A IMMUNOSUPPRESSANTS, present	-6.30	4.41	1.55E-01	1.00E+00
cDLCO, % pred	-0.09	0.07	1.74E-01	1.00E+00
R03A ADRENERGICS INHALANTS, present	-3.65	2.71	1.79E-01	1.00E+00
ITGV, l	-1.91	1.43	1.83E-01	1.00E+00
RDW, %	0.84	0.63	1.88E-01	1.00E+00
BORG max during 6MWD, score	0.75	0.57	1.88E-01	1.00E+00
LVEDD PSAX, mm	-0.22	0.17	1.95E-01	1.00E+00
N05B ANXIOLYTICS, present	7.98	6.14	1.96E-01	1.00E+00
Height, cm	0.20	0.15	1.98E-01	1.00E+00
KCO, % pred	-0.07	0.06	1.99E-01	1.00E+00
LAA, cm ²	0.26	0.20	1.99E-01	1.00E+00
A10B BLOOD GLUCOSE LOWERING DRUGS EXCL INSULINS, present	5.86	4.59	2.03E-01	1.00E+00
DBP, mmHg	0.11	0.08	2.07E-01	1.00E+00
WBC, 10 ⁶ /l	0.69	0.55	2.07E-01	1.00E+00
DM, present	4.26	3.41	2.13E-01	1.00E+00
RV, % pred	-0.04	0.03	2.15E-01	1.00E+00
FEV1, l	-2.29	1.88	2.25E-01	1.00E+00

HGB, g/dl	-0.76	0.62	2.25E-01	1.00E+00
BSA, m ²	10.82	9.21	2.42E-01	1.00E+00
HR max during 6MWD, bpm	-0.08	0.07	2.48E-01	1.00E+00
Last meal before sampling, hours	-0.29	0.25	2.48E-01	1.00E+00
TSH, mU/l	0.58	0.53	2.76E-01	1.00E+00
R03BA Glucocorticoids INHALANTS, present	-3.62	3.37	2.83E-01	1.00E+00
Smoking, never/past/current	-2.17	2.03	2.88E-01	1.00E+00
RV, l	-1.58	1.49	2.91E-01	1.00E+00
LVEF, %	-0.21	0.20	2.94E-01	1.00E+00
N05A ANTIPSYCHOTICS, present	-7.58	7.30	3.01E-01	1.00E+00
C10A LIPID MODIFYING AGENTS, present	2.79	2.86	3.31E-01	1.00E+00
Asthma, present	-3.89	4.13	3.48E-01	1.00E+00
J05 ANTIVIRALS FOR SYSTEMIC USE, present	-10.26	11.31	3.66E-01	1.00E+00
G03 SEX HORMONES AND MODULATORS OF THE GENITAL SYSTEM, present	-7.36	8.22	3.72E-01	1.00E+00
H03 THYROID THERAPY, present	-2.54	2.98	3.95E-01	1.00E+00
A11CC Vitamin D and analogues, present	-2.62	3.15	4.07E-01	1.00E+00
BE, mmol/l	-0.34	0.41	4.12E-01	1.00E+00
B03B VITAMIN B12 AND FOLIC ACID, present	-5.02	6.12	4.13E-01	1.00E+00
Alcohol consumption, present	2.20	2.82	4.36E-01	1.00E+00
A02BC Proton pump inhibitors, present	1.88	2.41	4.37E-01	1.00E+00
PaCO ₂ , mmHg	-0.16	0.21	4.40E-01	1.00E+00
RVEDD long, mm	-0.10	0.13	4.49E-01	1.00E+00
A03FA Propulsives, present	-8.51	11.31	4.53E-01	1.00E+00
C09C ANGIOTENSIN II RECEPTOR BLOCKERS, present	2.42	3.22	4.53E-01	1.00E+00
C08C SELECTIVE CALCIUM CHANNEL BLOCKERS WITH MAINLY VASCULAR EFFECTS, present	-2.27	3.15	4.73E-01	1.00E+00
R06A ANTIHISTAMINES FOR SYSTEMIC USE, present	5.09	7.25	4.84E-01	1.00E+00
C02 ANTIHYPERTENSIVES, present	6.42	9.28	4.90E-01	1.00E+00
N06A ANTIDEPRESSANTS, present	2.50	3.62	4.91E-01	1.00E+00
M04A ANTIGOUT PREPARATIONS, present	-2.78	4.08	4.96E-01	1.00E+00
R03BB Anticholinergics INHALANTS, present	-2.08	3.12	5.06E-01	1.00E+00
D shape of RV, present	1.67	2.56	5.15E-01	1.00E+00
E/A, arbitrary unit	-1.43	2.20	5.17E-01	1.00E+00
PAD, present	3.48	5.49	5.27E-01	1.00E+00
HCO ₃ , mmol/l	-0.22	0.36	5.47E-01	1.00E+00
B01AA Vitamin K antagonists, present	2.09	3.52	5.53E-01	1.00E+00
BORG min during 6MWD, score	0.57	0.98	5.66E-01	1.00E+00
L01E PROTEIN KINASE INHIBITORS, present	-3.48	6.15	5.72E-01	1.00E+00

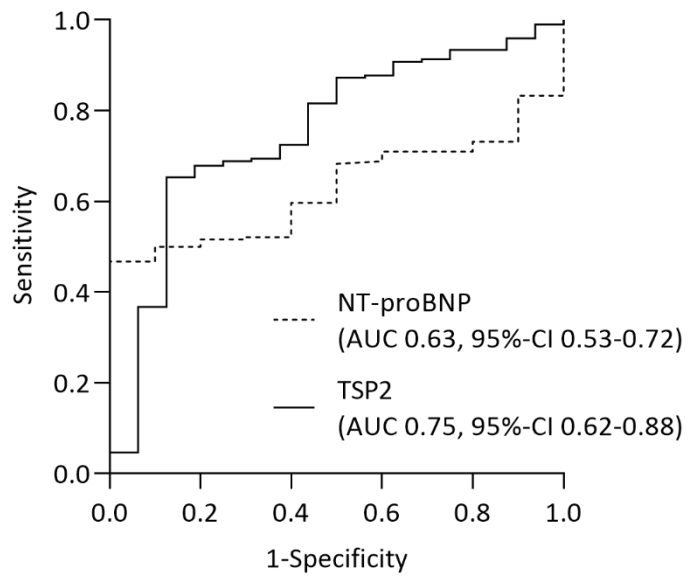
SBP, mmHg	-0.03	0.06	5.78E-01	1.00E+00
Malignancy in history or current, present	2.17	4.02	5.90E-01	1.00E+00
cDLCO, mmol/min/kPa	-0.36	0.73	6.23E-01	1.00E+00
Crea, mg/dl	0.30	0.63	6.32E-01	1.00E+00
PLT, 10 ⁶ /l	-0.01	0.02	6.42E-01	1.00E+00
BMI, kg/m ²	0.08	0.17	6.47E-01	1.00E+00
Septal bounce or RV, present	-1.17	2.67	6.62E-01	1.00E+00
SpO2 min during 6MWD, %	-0.07	0.16	6.62E-01	1.00E+00
N04 ANTI PARKINSON DRUGS, present	3.48	8.09	6.68E-01	1.00E+00
Bili, mg/dl	0.74	1.82	6.87E-01	1.00E+00
A10A INSULINS AND ANALOGUES, present	3.71	9.34	6.91E-01	1.00E+00
pH, arbitrary unit	12.72	36.14	7.25E-01	1.00E+00
MBP, mmHg	-0.03	0.08	7.41E-01	1.00E+00
COPD, present	1.00	3.08	7.46E-01	1.00E+00
KCO, mmol/min/kPa/l	-0.14	0.45	7.58E-01	1.00E+00
FEV1/FVC, %	-0.05	0.16	7.62E-01	1.00E+00
M01A ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS NON STEROIDS, present	-1.96	6.67	7.69E-01	1.00E+00
PaO2, mmHg	0.02	0.08	8.04E-01	1.00E+00
B03A IRON PREPARATIONS, present	-0.96	3.91	8.07E-01	1.00E+00
A11DA Vitamin B1 plain, present	-1.90	8.12	8.16E-01	1.00E+00
G04B UROLOGICALS, present	-1.32	7.23	8.56E-01	1.00E+00
B01AC Platelet aggregation inhibitors excl heparin, present	-0.57	3.45	8.68E-01	1.00E+00
B01AF Direct factor Xa inhibitors, present	0.39	2.65	8.84E-01	1.00E+00
HR min during 6MWD, bpm	-0.01	0.10	9.12E-01	1.00E+00
SpO2 max during 6MWD, %	0.04	0.43	9.24E-01	1.00E+00
CAD, present	-0.20	3.18	9.51E-01	1.00E+00
Hypertension, present	-0.17	2.94	9.53E-01	1.00E+00
C09A ACE INHIBITORS, present	0.15	3.12	9.62E-01	1.00E+00
Oxygen treatment, present	0.03	0.73	9.67E-01	1.00E+00
IVSd, mm	-0.02	0.46	9.69E-01	1.00E+00
C08D SELECTIVE CALCIUM CHANNEL BLOCKERS WITH DIRECT CARDIAC EFFECTS, present	-0.14	7.25	9.84E-01	1.00E+00
J02 ANTIMYCOTICS FOR SYSTEMIC USE, present	0.05	2.45	9.85E-01	1.00E+00
Vegetarianism or veganism, present	0.08	7.31	9.91E-01	1.00E+00

Supplementary Table 3: Results from linear regression models associating clinical measures with TSP2 levels in patients with pulmonary hypertension (PH), independent of age at sampling, body mass index (BMI), gender and gamma-glutamyl transferase (GGT) or estimated glomerular filtration rate (eGFR). Unadjusted p-values are presented for this secondary analysis.

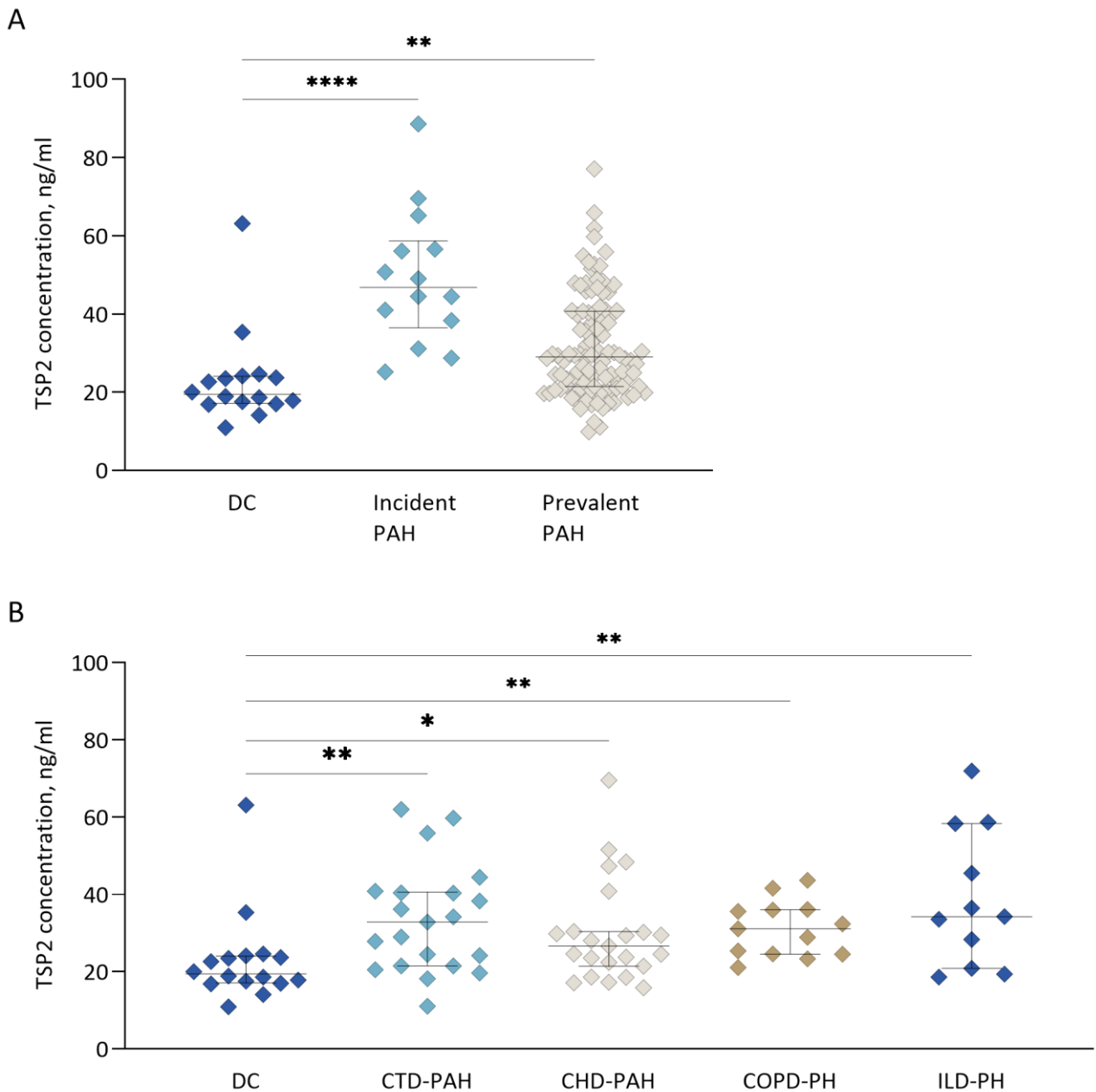
Clinical variable	In patient with PH (secondary analysis)		
	Estimate	SE	p-value
Model adjusted for age, gender, BMI and GGT			
MRAP, mmHg	1.23	0.26	5.37E-06
NT-proBNP, 100 ng/l	0.10	0.02	1.45E-04
RAA, cm ²	0.51	0.13	1.47E-04
RVFAC, %	-0.37	0.12	2.84E-03
TAPSE, mm	-0.68	0.25	7.15E-03
eGFR, ml/min	-0.19	0.05	2.01E-04
RVEDP, mmHg	0.82	0.23	5.18E-04
TAPSE/SPAP, 0.01 mm/mmHg	-0.27	0.09	3.49E-03
SVI, ml/m ²	-0.33	0.11	3.66E-03
SvO ₂ , %	-0.47	0.13	6.64E-04
SV, ml	-0.16	0.06	5.41E-03
Model adjusted for age, gender, BMI and eGFR			
GGT, UI/l	0.12	0.02	3.33E-08
MRAP, mmHg	1.27	0.25	9.20E-07
NT-proBNP, 100 ng/l	0.11	0.03	2.06E-04
RAA, cm ²	0.54	0.13	7.77E-05
RVFAC, %	-0.50	0.12	5.80E-05
TAPSE, mm	-0.95	0.25	1.75E-04
RVEDP, mmHg	0.81	0.21	2.23E-04
TAPSE/SPAP, 0.01 mm/mmHg	-0.38	0.09	3.55E-05
SVI, ml/m ²	-0.38	0.10	4.40E-04
SvO ₂ , %	-0.46	0.14	1.05E-03
SV, ml	-0.18	0.05	5.64E-04

Supplementary Table 4: Characteristics of patients with pulmonary arterial hypertension (PAH) and different right ventricular phenotypes (RV). A maladaptive RV phenotype was defined based on functional (CI, TAPSE) and structural criteria (RVEDA).

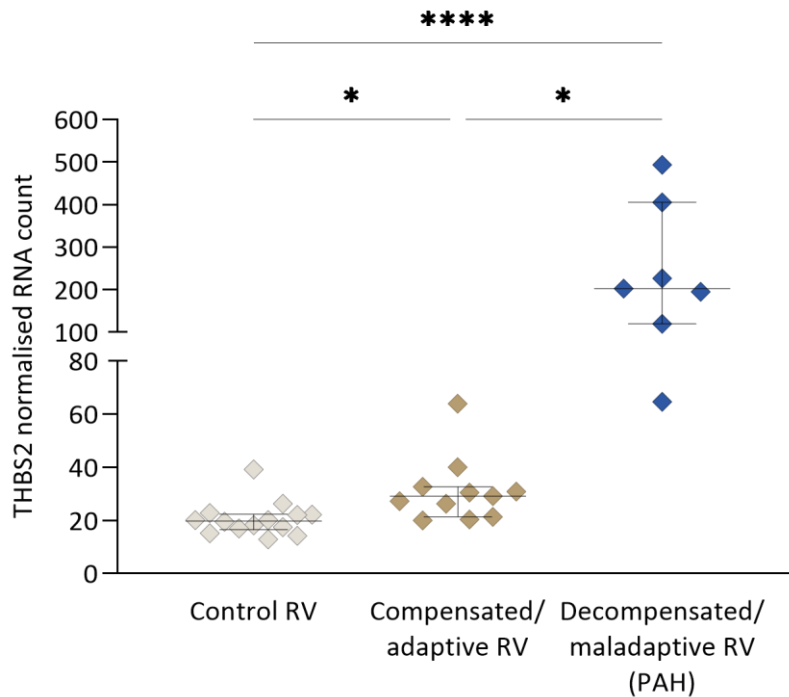
	RV phenotypes in PAH	
	Adaptive RV	Maladaptive
Subjects, N	90	10
Age, years	64 (44-74)	59 (53-69)
Female, %	71	50
BMI, kg/m ²	25 (22-29)	26 (24-30)
Exercise		
WHO-FC ≥III, %	41	60
6MWD, m	392 (284-468)	406 (257-460.5)
Right heart catheterisation		
sPAP, mmHg	62 (50.5-74)	84 (77-89)
mPAP, mmHg	24 (17-31)	36 (31-37)
dPAP, mmHg	39 (31-48)	54 (50-56)
mPAWP, mmHg	10 (6-13)	11 (6-13)
RAP, mmHg	6 (3.8-9)	10 (9.3-13.8)
CI, l/min/m ²	2.7 (2.3-3)	1.9 (1.8-2)
PVR, WU	5.8 (4.4-8.2)	10.4 (9-12)
Echocardiography		
RAA, cm ²	20 (15-25)	31 (24-40)
TAPSE, mm	19 (17-22)	13 (12-15)
TAPSE/sPAP, mm/mmHg	0.3 (0.2-0.4)	0.2 (0.1-0.2)
RVFAC, %	37 (32-42)	23.4 (20-25)
LAA, cm ²	17 (14-22)	11 (9-17)
LVEF, %	60 (56-63)	57.5 (55-60)
Pulmonary function test		
FEV1/FVC, %	0.7 (0.7-0.8)	0.8 (0.8-0.8)
TLC, % pred	89 (82-97)	80 (76-94)
Biochemistry		
TSP, mg/l	30 (23-41)	52 (48-65)
NT-proBNP, ng/l	675 (235-1459)	5290 (3059-6999)



Supplementary Figure 1: Receiver operating characteristics curve showing the discriminatory abilities of thrombospondin-2 (TSP2) and N-terminal pro-brain natriuretic peptide (NT-proBNP) to distinguish patients with pulmonary hypertension from controls. Area under the curve (AUC) and confidence interval (CI).



Supplementary Figure 2: Box and scatter plots comparing plasma thrombospondin-2 (TSP2) concentrations in patients with pulmonary arterial hypertension (PAH) with respect to time of sampling (at diagnosis/incident version during follow-up/prevalent; A), and in patients with different form of pulmonary hypertension (PH, B) and disease controls (DC) presenting with suspected PH but normal pulmonary artery pressure at cardiac catheterization. Boxes represent median and interquartile range. False discovery rate (FDR) procedure was applied to obtain q-value. Connective tissue disease (CTD), congenital heart disease (CHD), chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), $q < 0.05$ (*), $q < 0.01$ (**), $q < 0.0001$ (****).



Supplementary Figure 3: Box and scatter plots comparing thrombospondin-2 (*THBS2*) RNA count in right ventricular (RV) tissue obtained from controls (N=14), patients with compensated/adaptive RV function (N=11) and PAH patients with decompensated/maladaptive RV function (N=7). False discovery rate (FDR) procedure was applied to obtain q-value. RNA sequencing data were obtained from Boucherat O, et al. (Identification of LTBP-2 as a plasma biomarker for right ventricular dysfunction in human pulmonary arterial hypertension. *Nat Cardiovasc Res.* 2022;1:748–760. Gene Expression Omnibus repository identifier GSE198618). $q < 0.05$ (*) and $q < 0.0001$ (****).