Supplementary Materials

Circulating Markers of Inflammation and Angiogenesis and Clinical Outcomes Across Subtypes of Pulmonary Arterial Hypertension

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Contents

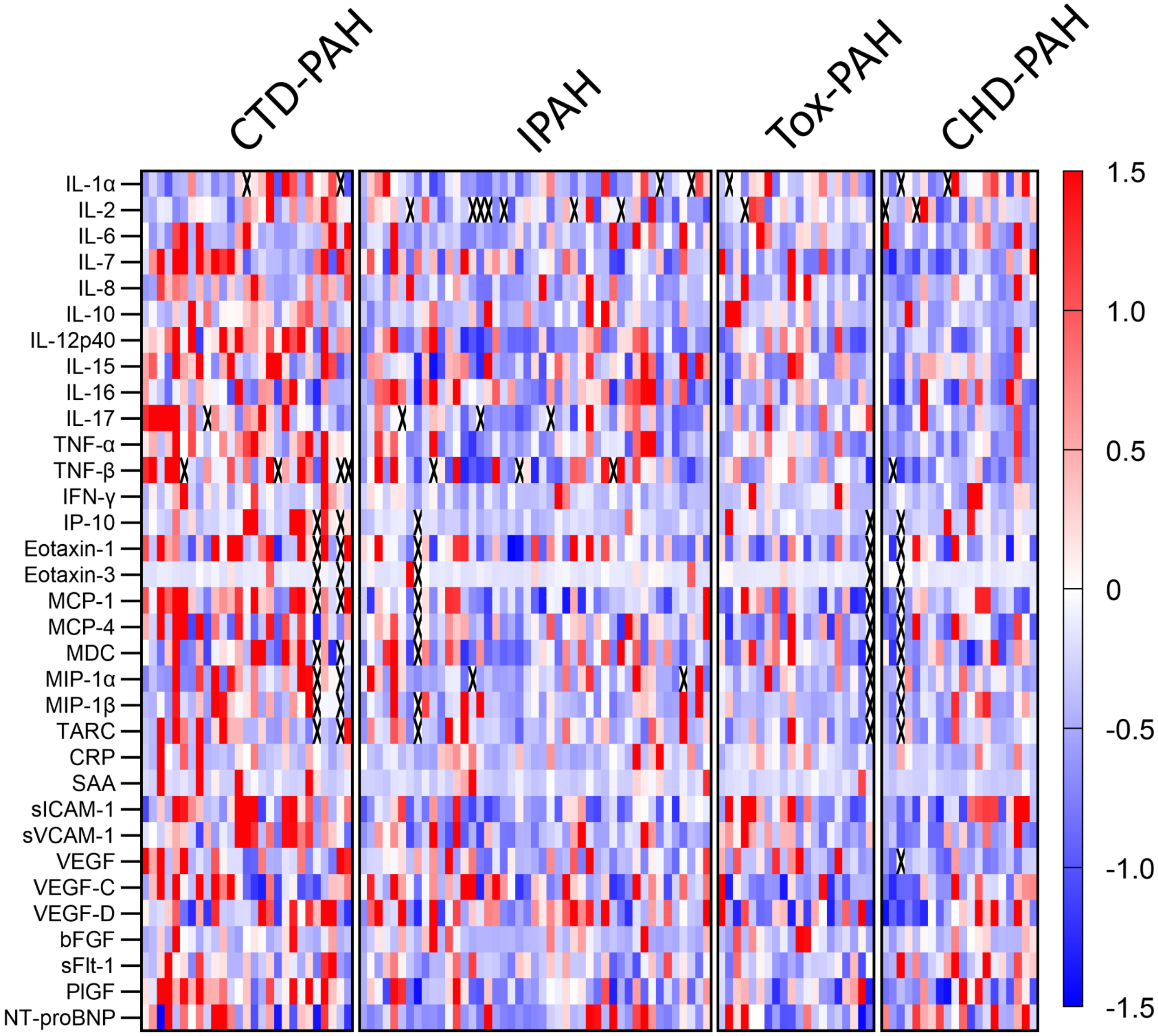


Figure S1: Heatmap of individual biomarker concentrations. Individually measured serum biomarkers were converted to row z-scores across all subtypes where each column represents a patient, and each row represents a biomarker. Patients are grouped by subtype. X-marked boxes indicate missing data. Definition of abbreviations: CTD-PAH: connective tissue disease-associated pulmonary arterial hypertension; IPAH: idiopathic pulmonary arterial hypertension; Tox-PAH: toxin-associated pulmonary arterial hypertension; CHD-PAH: congenital heart disease-associated pulmonary arterial hypertension.

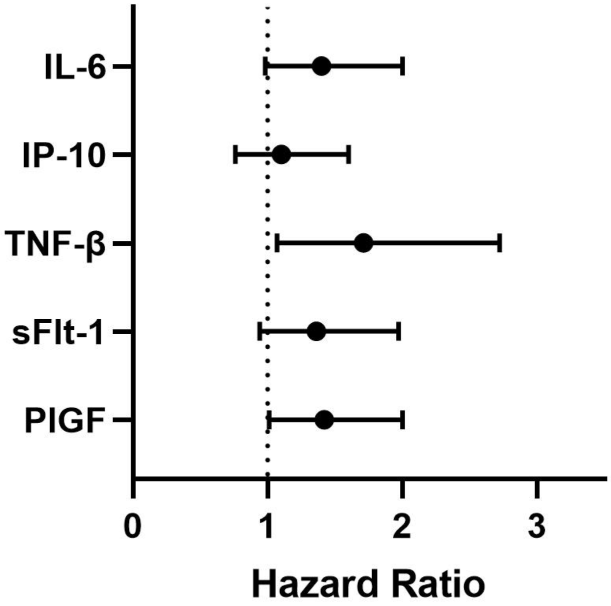
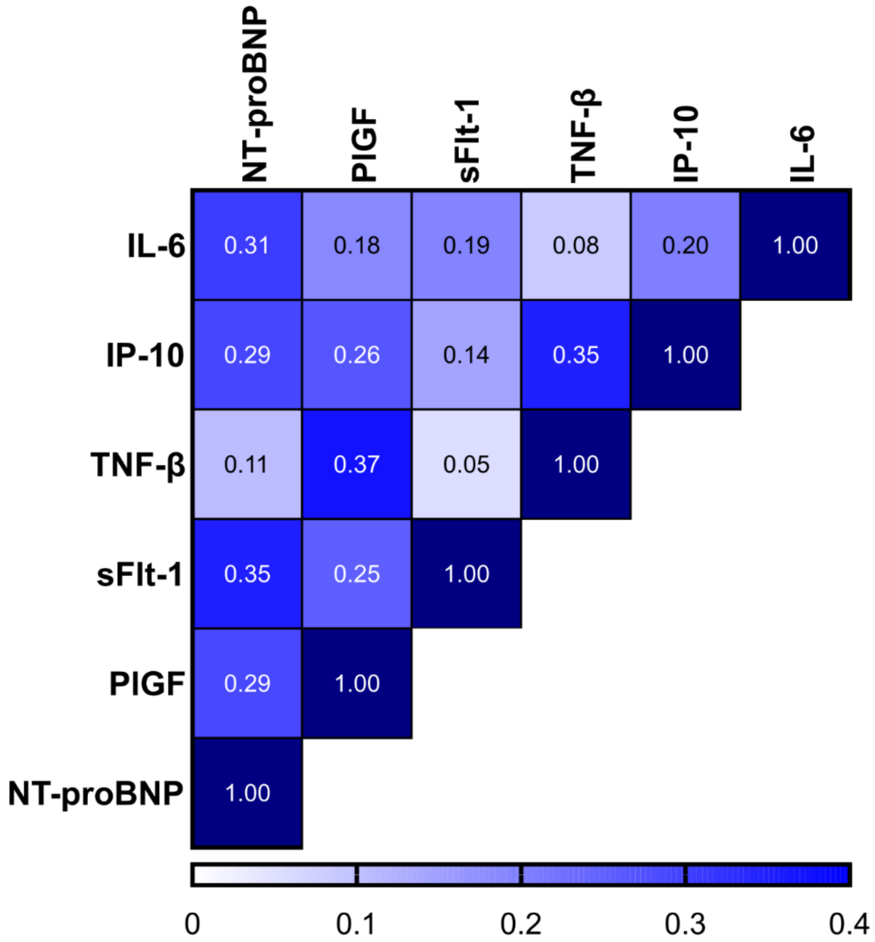


Figure S2: Biomarker association with mortality with additional adjustment for NT-proBNP. Cox proportional hazards model from the main text (Figure 2) with additional post hoc adjustment for NT-proBNP in addition to original adjustments of age, sex, and pulmonary arterial hypertension etiology. Graph shows the hazard ratio for a biomarker standard deviation change against mortality in the pooled PAH cohort. IL-6: interleukin-6; IP-10: interferon gamma-induced protein 10; TNF-β: tumor necrosis factor-beta; sFlt-1: soluble fms-like tyrosine kinase 1; PlGF: placental growth factor; NT-proBNP: N-terminal pro-brain natriuretic peptide.



	NT-proBNP	PlGF	sFlt-1	TNF-β	IP-10	IL-6
IL-6	0.31	0.18	0.19	0.08	0.20	1.00
IP-10	0.29	0.26	0.14	0.35	1.00	
TNF-β	0.11	0.37	0.05	1.00		
sFlt-1	0.35	0.25	1.00			
PlGF	0.29	1.00				
NT-proBNP	1.00					

Figure S3: Correlation among mortality-associated biomarkers. Spearman's rank correlation coefficients were determined across biomarkers associated with mortality in the overall PAH cohort. Shading reflects the relative strength of correlation as indicated by the key at the bottom of the image.

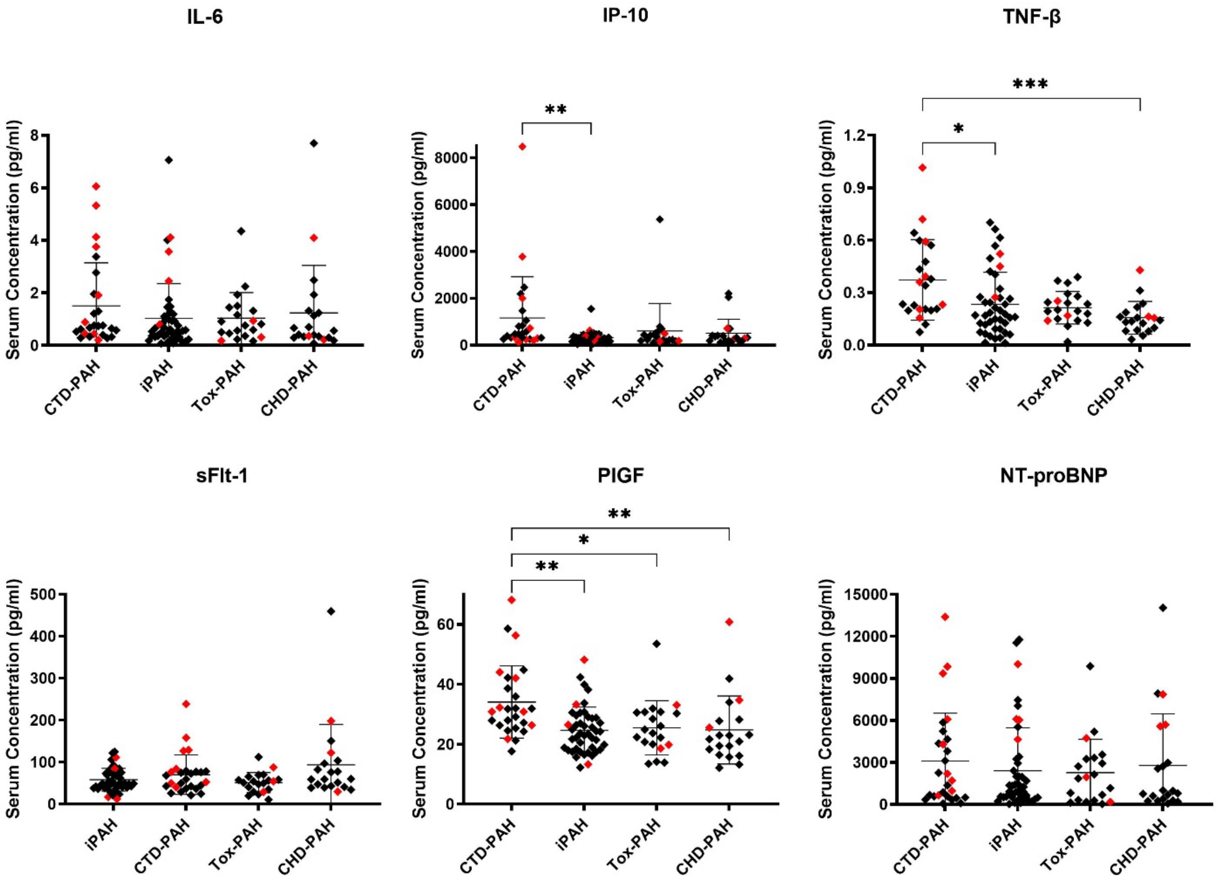


Figure S4: Concentration of mortality-associated biomarkers by subtype. Dot plot displays biomarker concentrations for each patient, by PAH subtype, with bars denoting mean and standard deviation. A red dot signifies non-surviving patients at three years, while black dot signifies surviving patients. Significant differences between groups were determined with Kruskall Wallis one-way analysis of variance with Dunn's multiple comparisons (* p<0.05, ** p<0.01, *** p<0.001). Definition of abbreviations: PAH: pulmonary arterial hypertension; IPAH: idiopathic PAH; CTD-PAH: connective tissue disease-associated PAH; tox-PAH: toxin-associated PAH; CHD-PAH: congenital heart disease-associated PAH; IL-6: interleukin-6; IP-10: interferon gamma-induced protein 10; TNF-β: tumor necrosis factor-beta; sFlt-1: soluble fms-like tyrosine kinase 1; PlGF: placental growth factor; NT-proBNP: N-terminal pro-brain natriuretic peptide.

Table S1: Biomarker associations with five-year survival in the UK PAH Cohort Study

Target Protein	SomaScan SeqId	UniProt	Hazard Ratio	p-value
sFlt-1	8231-122	P17948	1.07	0.269
sFlt-1	16315-105	P17948	1.17	0.111
PlGF	3078-1	P49763	1.19	0.050
IP-10	4141-79	P02778	1.18	<0.001
IL-6	4673-13	P0523	1.03	0.582
TNF-β	4703-87	P01374	1.19	0.009
NT-proBNP	7655-11	P16860	1.58	<0.001

As focused validation of the six biomarkers associated with mortality in the SeRVeTuS cohort, Cox regression analyses adjusted for age and sex were performed on aptamer-based proteomic data (SomaScan 4) from the UK PAH Cohort Study. Biomarker levels were related to five-year mortality or lung transplant. Two aptamers for sFLT-1 were available, and both were individually analyzed. Four markers associated significantly with worse transplant-free survival at a significance threshold of $p \leq 0.05$: PlGF, IP-10, TNF-β, and NT-proBNP.

Table S2: Biomarker associations with echocardiographic and clinical outcomes across subtypes

A. All PAH (Pooled Cohort)																
	REVEAL 2.0 score change				**RVD change (mm)**				**TAPSE change (mm)**				**6MWD (meter) change**			
	unadjusted		adjusted		unadjusted		adjusted		unadjusted		adjusted		unadjusted		adjusted	
	score	p-value	score	p-value	mm	p-value	mm	p-value	mm	p-value	mm	p-value	m	p-value	m	p-value
sFlt-1	0.45	0.06	0.46	0.03	-1	0.13	-1.0	0.23	0	0.48	0	0.74	-7.0	0.52	-8.5	0.43
PlGF	1.17	<0.001	0.66	0.004	-1	0.13	-1.0	0.49	0	0.57	0	0.85	-35.7	0.001	-30.5	0.02
IP-10	0.66	0.006	0.44	0.05	1	0.09	1.0	0.08	-1	0.11	-1	0.33	-21.3	0.05	-21.0	0.06
TNF-β	0.60	0.014	0.12	0.61	1	0.37	1.0	0.41	1	0.19	1	0.22	-5.5	0.61	2.1	0.86
IL-6	0.73	0.001	0.57	0.004	1	0.18	2.0	0.04	1	0.17	1	0.39	-35.7	0.001	-29.6	0.005
BNP	1.43	<0.001	1.32	<0.001	4	<0.001	5.0	<0.001	-2	0.002	-2	0.004	-60.0	<0.001	-68.6	<0.001
B. CTD-PAH																
	REVEAL 2.0 score change				**RVD change (mm)**				**TAPSE change (mm)**				**6MWD (meter) change**			
	unadjusted		adjusted		unadjusted		adjusted		unadjusted		adjusted		unadjusted		adjusted	
	score	p-value	score	p-value	mm	p-value	mm	p-value	mm	p-value	mm	p-value	m	p-value	m	p-value
sFlt-1	1.28	0.013	1.17	0.02	-1	0.75	0.0	0.93	-1	0.44	-1	0.42	-25.3	0.31	-22.9	0.36
PlGF	0.52	0.22	0.54	0.20	-2	0.18	-2.0	0.29	0	0.69	0	0.99	-35.7	0.07	-33.5	0.08
IP-10	0.52	0.094	0.54	0.08	2	0.08	2.0	0.06	-1	0.14	-1	0.26	-21.9	0.11	-29.0	0.02
TNF-β	0.11	0.80	0.14	0.75	1	0.63	4.0	0.28	0	0.66	1	0.63	7.6	0.71	-30.8	0.21
IL-6	0.60	0.163	0.53	0.21	1	0.26	2.0	0.14	0	0.85	0	0.64	-60.4	0.001	-56.1	0.001
BNP	1.13	0.008	1.07	0.01	3	0.05	5.0	0.002	-1	0.13	-1	0.18	-51.8	0.009	-56.4	0.002
C. Non-CTD-PAH																
	REVEAL 2.0 score change				**RVD change (mm)**				**TAPSE change (mm)**				**6MWD (meter) change**			
	unadjusted		adjusted		unadjusted		adjusted		unadjusted		adjusted		unadjusted		adjusted	
	score	p-value	score	p-value	mm	p-value	mm	p-value	mm	p-value	mm	p-value	m	p-value	m	p-value
sFlt-1	0.20	0.41	0.29	0.2	-1	0.17	1	0.15	0	0.62	0	0.91	-1.8	0.87	-5.5	0.67
PlGF	1.19	<0.001	0.81	0.008	0	0.88	0	0.81	0	0.87	-1	0.48	-31.4	0.03	-31.7	0.08
IP-10	0.19	0.65	0.23	0.55	-2	0.18	0	0.99	-1	0.28	0	0.97	-5.8	0.78	-4.6	0.83
TNF-β	0.35	0.25	0.12	0.67	2	0.22	1	0.62	1	0.09	1	0.17	-0.6	0.96	-5.5	0.72
IL-6	0.63	0.013	0.60	0.01	2	0.17	2	0.07	1	0.13	1	0.2	-21.9	0.1	-17.7	0.2
BNP	1.45	<0.001	1.42	<0.001	4	<0.001	5	<0.001	-2	0.005	-2	0.02	-62.2	<0.001	-77.7	<0.001

Linear regression models were run on biomarker associations with REVEAL 2.0 score, right ventricle diameter (RVD), tricuspid annular plane systolic excursion (TAPSE), and 6-minute walk distance (6MWD). Models were performed **A.** on the entire cohort; **B.** on the subset of connective tissue disease patients (CTD-PAH); and **C.** on the non-CTD-PAH subgroup (patients with idiopathic, congenital heart disease-associated, and toxin-associated PAH). Data are shown from both unadjusted models, and models adjusted for age, sex, etiology, and height. The change in REVEAL 2.0 score, RVD, TAPSE, or 6MWD associated with a standard deviation change in biomarker concentration is shown.