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TITLE PAGE

Angiopoietin 2 and hsCRP are associated with pulmonary haemodynamics and long-term mortality respectively in CTEPH - results from a prospective discovery and validation biomarker study

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ABSTRACT

Chronic thromboembolic pulmonary hypertension (CTEPH) is an underdiagnosed disease of uncertain aetiology. Altered endothelial homeostasis, defective angiogenesis and inflammation are implicated. Angiopoietin 2 (Ang2) impairs acute thrombus resolution and is associated with vasculopathy in idiopathic pulmonary arterial hypertension.

We assessed circulating proteins associated with these processes in serum from patients with CTEPH (n = 71) before and after pulmonary endarterectomy (PEA), chronic thromboembolic pulmonary disease without pulmonary hypertension (CTEPD, n = 9) and healthy controls (n = 20) using Luminex multiplex arrays. Comparisons between groups were made using multivariable rank regression models. Angiopoietin 2 (Ang2) and high-sensitivity C-reactive protein (hsCRP) were measured in a larger validation dataset (CTEPH = 277, CTEPD = 26). Cox proportional hazards models were used to identify markers predictive of survival.

In CTEPH patients, Ang2, interleukin (IL) 8, tumour necrosis factor α, and hsCRP were elevated compared to controls, while vascular endothelial growth factor (VEGF) c was lower ($p < 0.05$). Ang2 fell post PEA ($p < 0.05$) and was associated with both pre- and post-PEA pulmonary haemodynamic variables and functional assessments ($p < 0.05$). In the validation dataset, Ang2 was significantly higher in CTEPH compared to CTEPD. Pre-operative hsCRP was an independent predictor of mortality.

68

69 We hypothesise CTEPH patients have significant distal micro-vasculopathy and consequently
70 high circulating Ang2. Patients with CTEPD without pulmonary hypertension have no
71 discernible distal micro-vasculopathy and therefore have low circulating Ang2. This suggests
72 Ang2 maybe critical to CTEPH disease pathogenesis (impaired thrombus organisation and
73 disease severity).

74

75

INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is an underdiagnosed type of pulmonary hypertension (PH) and has a poorly understood pathobiology [1,2]. The three-compartment model of CTEPH describes occlusion of proximal and subsegmental pulmonary arteries by organized chronic thromboembolic material and a distal pulmonary vasculopathy due to remodelling of small unobstructed pulmonary arteries [3-5]. The formation of the organized occlusions is considered to arise from a failure to resolve acute pulmonary emboli. The vasculopathy is thought to be a secondary response to alterations in blood flow distribution caused by the proximal chronic thromboembolic material or increased flow through bronchial collaterals [6,7]. Both the occlusions and vasculopathy contribute to an elevated pulmonary vascular resistance (PVR), which in turn leads to right ventricular (RV) failure, the commonest cause of morbidity and mortality in all forms of PH [8,9].

Emerging evidence supports the role of mediators of angiogenesis, inflammation and the coagulation cascade (and their many interactions) in CTEPH and idiopathic pulmonary arterial hypertension (IPAH) pathogenesis [10-19]. Angiopoietin 2 (Ang 2) was recently shown to be elevated in CTEPH and impaired venous thrombus resolution in a mouse model [20]. It is also elevated in IPAH and predictive of death in this patient group [10]. This suggests that Ang2 is also a marker of distal vasculopathy and endothelial dysfunction; processes that are similar in the two diseases.

We hypothesised that mediators of angiogenesis and inflammation are associated with clinical markers of disease severity and outcomes. In comparison to previous studies, we assessed a wide range of circulating proteins in a large cohort, allowing use of more robust multivariable modelling to assess their role in disease pathogenesis and potential clinical implications.

METHODS

Study design

Consecutive patients undergoing pulmonary endarterectomy (PEA) at Royal Papworth Hospital, UK, who consented for participation in the study 'Molecular mechanisms of right ventricular adaption to increased afterload' (regional ethics reference: 13/EE/0331), and the Research Tissue Bank (08/H0304/56+5) between February 2012 and August 2014, were recruited. A validation data set included all consecutive samples collected from CTEPH patients prior to PEA between April 2010 and Aug 2017 (samples represented in the discovery group were excluded in the validation analyses).

Study subjects

Patients diagnosed with CTEPH (mean pulmonary artery pressure [mPAP] ≥ 25 mmHg, n = 71) according to international guidelines were recruited [21]. Patients with chronic thromboembolic

occlusions without pulmonary hypertension (CTEPD; mPAP < 25 mmHg, n = 9) were included as a disease comparator, because their disease is thromboembolic in origin but they have not developed pulmonary hypertension at rest. Twenty age and sex matched volunteers were recruited for comparison. A further 20 incident patients (not on pulmonary artery vasodilator therapies) with IPAH were included as an additional disease comparator. The validation dataset included 277 CTEPH patients and 26 CTEPD patients without PH, who had serum samples biobanked pre-PEA in our Tissue Bank.

Serum collection and biomarker measurement

CTEPH and CTEPD patients had serum samples collected from a peripheral vein pre-PEA (either at the time of initial right heart catheterisation or on admission prior to PEA [for patients referred for surgery from other UK National Pulmonary Hypertension Centres]). In a subset of patients, further sampling was performed at the first follow up visit to Papworth Hospital 3-6 months post-PEA. This time point minimizes perioperative changes that may also influence circulating protein levels. Healthy volunteer control and IPAH samples were processed according to the same protocol.

Systemic levels of cytokines and angiogenic proteins were measured in duplicate wells using either a customized human cytokine/chemokine magnetic bead assay (IL6, IL8, IL10, TNFα) or a customized human angiogenesis/growth factor assay (angiopoietin 2 [Ang2], endoglin,

vascular endothelial growth factor [VEGF] a, VEGFc, VEGFd and bone morphogenetic protein 9 [BMP9]; Millipore, UK). Pre-PEA and post-PEA samples for each patient were placed on adjacent wells on the same plates. high-sensitivity C-Reactive Protein (hsCRP) and Ang2 were measured in a clinical pathology laboratory in pre-PEA samples. hsCRP was measured using latex enhanced immunoturbidimetric assay on the Siemens Dimension EXL Autoanalyser. Ang2 was measured using the MesoScale Discovery electrochemiluminescence immunoassay on specially formatted microtitre plates.

In the validation dataset, hsCRP and Ang2 were measured in the same clinical pathology laboratory using the platforms described above.

Statistical analysis

Statistical analyses were performed using R (www.r-project.org). Rank based regressions were used to analyse the data [22]. These regression coefficients can be interpreted in the same way as in a linear model. Cox-proportional hazards models were used to assess survival. Please see the online supplement for more details.

Results

Subject characteristics

The baseline characteristics of patients recruited to the discovery arm are presented in Table

1 and in the online supplement.

Angiopoietin 2 is elevated in CTEPH compared to healthy controls

First, we compared the differences in circulating serum proteins in the disease groups to the healthy control group. Rank regression models were used to assess the association of serum protein levels with disease state (CTEPH Pre PEA, CTEPD Pre PEA and control; Figure 1 and Supplementary Table 2). Age, gender and batch were used as covariables in the analysis. Ang2, IL8, TNFα, and hsCRP were significantly elevated in CTEPH patients compared to the control group (all $p_{corrected} < 0.05$). VEGFc was lower in CTEPH patients ($p_{corrected} < 0.05$). Whereas, in CTEPD patients IL10 was significantly higher compared to the control group ($p_{corrected} < 0.05$), although there were only 9 patients with CTEPD.

To assess the contribution of the organising chronic thromboembolic material and the distal vasculopathy to changes in circulating proteins we compared differences between the CTEPH group (with organising chronic thromboembolic material and distal vasculopathy), and the 2 disease comparator groups (CTEPD [with organising chronic thromboembolic material and less/no distal vasculopathy] and IPAH [with distal vasculopathy but no organising chronic thromboembolic material]).

In rank regression models (PVR, age, gender and batch used as covariables) no significant

differences were seen in Ang2 levels between CTEPH patients and either CTEPD patients or IPAH patients (Supplementary Table 3). CTEPH patients had lower VEGFd levels compared to IPAH ($p_{corrected} < 0.05$).

Given there were only 9 patients with CTEPD without PH in the discovery dataset, a further comparison between CTEPH and CTEPD was made in the validation dataset (see below).

Ang2 levels fell significantly following PEA surgery

Changes to circulating serum proteins following PEA surgery were assessed. Serum protein levels were measured in 47 CTEPH patients pre- and post-PEA. Significant improvements in haemodynamic and functional assessments were observed post-PEA (Table 2). Significant reductions in Ang2, IL8, IL10, VEGFc, VEGFd, and endoglin were observed (Table 2, $p_{corrected} < 0.05$). There was a significant increase in VEGFa ($p_{corrected} = 0.02$).

In comparison with controls, the CTEPH post-PEA group had significantly higher Ang2 and TNFα levels ($p_{corrected} < 0.05$; Figure 2), as well as significantly reduced VEGFc and VEGFd levels ($p_{corrected} < 0.001$).

Ang2 was consistently associated with pulmonary haemodynamic measurements and NTproBNP

196 Associations between the serum proteins and pulmonary haemodynamic measurements,
197 clinical blood tests and functional assessments were assessed in CTEPH patients pre-PEA. Only
198 Ang2, sampled pre-operatively, showed a significant and consistent association with clinical
199 assessments recorded at baseline. Pre-PEA Ang2 was associated with pre-PEA mPAP, PVR, N-
200 terminal pro brain natriuretic peptide (NT-ProBNP; a marker of RV dysfunction) and functional
201 class (all $p_{corrected} < 0.001$; Figure 3). It was also inversely associated with pre-PEA CI ($p_{corrected}$
202 < 0.001).
203
204 Pre-PEA endoglin was also associated with pre-PEA PVR ($p_{corrected} < 0.001$) and NT-ProBNP
205 ($p_{corrected} < 0.001$), as well as inversely associated with pre-PEA 6mwt distance ($p_{corrected} =$
206 0.029).
207
208 <u>Pre-PEA Ang2 levels were significantly associated with post-PEA PVR</u>
209 Rank regression models were used to assess if serum proteins sampled before PEA could
210 predict clinical assessments made at follow-up 3 – 6 months post PEA. The only protein,
211 sampled preoperatively, that was associated with post-PEA clinical assessments was Ang2.
212 Pre-PEA Ang2 was significantly associated with post-PEA PVR (estimated regression
213 coefficient = 248, standard error = 66, $p_{corrected} < 0.001$).
214
215 <u>Validation dataset</u>

Ang2 was measured in a further 303 patients (277 with CTEPH and 26 patients with CTEPD without PH) to validate the key findings described above and further investigate the significance of these biomarkers in CTEPH. PEA was performed in 72% of CTEPH patients and 27% of CTEPD patients. The clinical characteristics of these patients are provided in Supplementary Table 4.

hsCRP was also assessed as a measure of inflammation and to validate the association between hsCRP and mortality reported by Skoro-Sajer et al [23]. hsCRP and Ang2 were strongly associated in a multivariable model including age, sex and PVR as covariables (estimated regression coefficient = 0.17, standard error = 0.03, $p < 0.001$).

Ang2 and hsCRP levels were significantly higher in CTEPH patients compared to controls in rank regression models using age and gender as covariables (Supplementary Table 5). Importantly, Ang2 was significantly lower in patients with CTEPD without PH compared to CTEPH patients ($p < 0.001$). Median (IQR) values for Ang2 were: control 4094 pg/ml (3457 - 5180), CTEPD 4554 pg/ml (3408 - 5575) and CTEPH 8197 pg/ml (4995 - 16851).

Significant associations between pre-PEA Ang2 and pre-PEA mPAP, PVR, CI and 6mwt distance were once again demonstrated in the validation dataset (Supplementary Table 6). Additionally, pre-PEA Ang2 was again significantly associated with post-PEA PVR (rank

236 regression model using age and gender as covariables: estimated regression coefficient = 7.9,
237 standard error = 2.3, p = 0.008).
238
239 To ascertain the clinical utility of this last observation, the ability of Ang2 to determine
240 significant residual PH post-PEA (defined as a PVR > 425 dynes [24]) was assessed using logistic
241 regression models. Clinical assessments for 166 patients, 3-6 months post-PEA, were
242 available. 42 patients had residual PH post-PEA. In univariable logistic regression modelling
243 pre-PEA Ang2 was a significant predictor of residual PH (regression coefficient = 4.5×10^{-5},
244 standard error = 2.2×10^{-5}, p = 0.038). However, it was not an independent predictor of residual
245 PH in logistic regression models including baseline PVR as a covariable.
246
247 Lastly, the associations between pre-PEA Ang2 and hsCRP and survival from the time of
248 sampling were assessed amongst the 199 patients undergoing PEA. 13 deaths were observed.
249 In univariable Cox proportional hazards models age, 6mwt distance and hsCRP were
250 associated with all-cause mortality (Supplementary Table 7). Pre-PEA haemodynamic
251 assessments and Ang2 were not significantly associated with survival. In a multivariable
252 model, hsCRP was associated with all-cause mortality in patients with CTEPH undergoing PEA
253 (Table 3).
254
255 **DISCUSSION**

In this study of serum biomarkers in CTEPH we investigated two pathobiological processes previously associated with pulmonary hypertension and impaired thrombus resolution. We show that Ang2 is increased in patients with CTEPH compared to both healthy controls and, importantly, patients with CTEPD without pulmonary hypertension. Ang2 is associated with pulmonary haemodynamics and falls following PEA, as recently revealed by Hobohm et al [20]. We show that hsCRP is an independent prognostic marker of survival in this patient group, supporting data from Skoro-Sajer et al [23]. Our data also suggests other molecules such as endoglin, IL10 and VEGF molecules may be differentially expressed in CTEPH compared to controls and other disease states.

Hobohm et al. demonstrated that Ang2 plays a critical role in CTEPH pathogenesis; by impairing thrombus resolution. In an extensive body of work, they describe high levels of Ang2 in organising PEA specimens and show Ang2 impairs venous thrombus resolution in two different models [20]. They suggest that Ang2 levels are higher in CTEPH patients compared to IPAH patients. Previously, Kümpers et al. had shown Ang2 expression is increased in plexiform lesions from patients with IPAH and found Ang2 to be an independent prognostic marker of survival in this group [10].

Our data validates aspects of the study from Hobohm et al. and makes new observations that extends our knowledge of Ang2 in CTEPH. The multivariable statistical analysis is a strength

of our study. We hypothesise that Ang2 is a marker of distal vasculopathy and/or endothelial dysfunction. Supporting this Ang2 is highest in CTEPH patients, as they have the most vasculopathy, and lowest in controls, as they have no vasculopathy. While patients with CTEPD without PH, may have no discernible vasculopathy and therefore low circulating Ang2.

We observed Ang2 expression associated with endothelialized channels within the chronic thromboembolic material (Supplementary Figure 2). Hobohm et al., have also shown increased expression of Ang2 in PEA specimens. However, we show that patients with CTEPD without PH had near normal circulating levels of Ang2, significantly lower than CTEPH patients, despite the presence of proximal chronic thromboembolic material. This suggests that the obstructive chronic thromboembolic material is not the *predominant* source of circulating Ang2 in CTEPH. We suggest that the source of elevated circulating Ang2 is more likely pulmonary endothelial dysfunction, analogous to previous studies in IPAH. We show that, if PVR is considered, there is no difference in Ang2 between CTEPH and IPAH groups. We cannot exclude the right ventricle as a source of Ang2 but to the best of our knowledge Ang2 is not expressed in cardiac myocytes. Our study and that of Hobohm et al., demonstrates the importance of Ang2 in disease pathogenesis, both in the initiation of disease (impaired thrombus resolution) and severity of disease (distal vasculopathy).

Ang2 was consistently associated with worse haemodynamic and functional assessments, like

296 that seen in IPAH. Baseline measurements of Ang2 was even associated with post PEA PVR,
297 although this association was not strong enough to be of clinical utility.
298
299 Endothelial cell integrity and function have been shown to be important in pulmonary arterial
300 hypertension and there is a growing body of evidence for its role in CTEPH pathobiology
301 [14,18,25-27]. Ang2 is a pleiotropic protein stored within Weibel Palade bodies of endothelial cells;
302 and whose function is dependent on the local extracellular milieu [28,29]. Ang2 antagonism of
303 TIE2/TEK2 serves to destabilize the endothelium while integrin binding leads to endothelial
304 cell sprouting and migration [28,30]. It promotes angiogenesis in the presence of VEGFa.
305
306 Angiogenesis is also critical to thrombus resolution [20,31]. In CTEPH, defective angiogenesis has
307 been linked to impaired thrombus resolution, the formation of chronic thromboembolic
308 material and associated with poor outcomes [16,18-20,32]. The extensive bronchial
309 collateralisation, sparsity of plexiform lesions and venous remodelling, characteristic of
310 CTEPH compared to other forms of PH, may be driven by the differential expression of
311 angiogenic molecules in CTEPH [7]. In vitro, Naito et al. show endothelial cells extracted from
312 chronic thromboembolic material demonstrate a high angiogenic potential [25]. Our data,
313 showing alterations in circulating Ang2, VEGF molecules and endoglin provide additional
314 mechanisms by which these histological changes may arise. Further research into the role of
315 the VEGF isoforms in PH is required. We are unaware of changes in lymphatic drainage in

316 patients with CTEPH.

317

318 Inflammation and angiogenesis are intricately related [33]. There is evidence to support the
319 importance of both these processes in pulmonary vascular homeostasis and the pathogenesis
320 of CTEPH. Wynants et al. demonstrated CRP increased the proliferation of pulmonary artery
321 smooth muscle cells from CTEPH patients [14]. More recently Skoro-Sajer et al. show that CRP
322 was a significant prognostic marker in CTEPH [23]. In our study, hsCRP was of prognostic
323 significance and closely associated with Ang2. Furthermore, IL8 was increased in patients with
324 CTEPH and fell post PEA. IL8 may be secreted by inflammatory cells recruited into the chronic
325 thromboembolic material or even from endothelial cells. IL8 has been implicated in
326 endothelial dysfunction and is a promoter of angiogenesis. These findings further support the
327 notion that inflammation and angiogenesis are linked and are key factors in the development
328 of endothelial dysfunction in CTEPH.

329

330 We also show that inflammatory cytokines (IL10 and TNFα) are elevated in CTEPH and fall
331 post PEA in keeping with previous studies. This has previously been attributed to
332 improvements in right ventricular function following PEA, however we did not see any
333 associations with pulmonary haemodynamics in rank regression models for these proteins [13].

334

335 Patients with CTEPD can be a useful comparator group to understand disease

pathophysiology and tease out from which “compartment” differences may arise, as they do not fulfil the haemodynamic criteria for PH and do not have clinically overt heart failure. Although, we have previously demonstrated these patients do not have entirely normal cardiac physiology [34]. This group of patients have not been well characterized, but with the growing recognition of a spectrum of disease referred to as post PE syndrome this is an important avenue for future research [35,36].

The study has several limitations. There are limited numbers of patients with CTEPD. Much larger numbers overall are needed to assess the potential interactions with confounders such pulmonary artery vasodilator therapies and different forms of anticoagulation. We have not assessed Ang2 expression in areas of vasculopathy to prove this is a source of circulating Ang2. However, we believe the vasculopathy in CTEPH is similar to that seen in IPAH. Kümpers et al. showed that Ang2 was upregulated in areas of vasculopathy [10]. Experimental models of CTEPH do not fully recapitulate human disease; therefore large, well-controlled observational studies are required to further our understanding of disease pathogenesis. The study has identified several interesting signals, such as differences in VEGF molecules and IL10 between patients with CTEPD and CTEPH. Further studies are required to validate them and assess their significance.

Summary

By targeted screening of key proteins related to inflammation, vascular homeostasis and angiogenesis, we provide useful insights into CTEPH pathobiology. IL8, IL10, TNFα and VEGF molecules are altered in CTEPH and may play a part in disease pathogenesis. Circulating levels of Ang2 are associated with pulmonary haemodynamics and Ang2 may be a marker of distal pulmonary circulation remodelling. Ang2 may have a role in assessing future treatments that affect distal pulmonary artery remodelling/vasculopathy in CTEPH and other forms of PH. Finally, we demonstrate that hsCRP is an independent prognostic marker in patients with CTEPH undergoing PEA. This study adds to an increasing body of evidence that the interplay between inflammatory cell signalling (a key trigger event in many forms of PH) and the subsequent angiogenic response to insult may play a key role in disease development.

DISCLOSURES

None

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AUTHOR CONTRIBUTIONS

CH, MS, MT, JPZ – designed the study, analysed the data and wrote the manuscript

JHS, MN, SP – analysed the data and contributed to writing the manuscript

KB, ES – collected data for the study and analysed the data

JC, KS, DT, NS, DJ, NWM – interpreted the data and contributed to writing the manuscript

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394 **TABLES**

Group	CTEPH Pre PEA	CTEPD Pre PEA	Control
n	71	9	20
Gender [% female]	39 [55%]	4 [44%]	12 [60%]
Age (years)	64 [52 - 71]	52 [35 - 69]	61 [58 - 62]
On pulmonary vasodilator therapy [%]*	39 [55%]	0 [0%]	0 [0%]
mPAP (mmHg)	45 [36 - 52]	22 [19 - 24]	
PAWP (mmHg)	12 [9 - 13]	10 [8 - 11]	
PVR (WU)	8.2 [4.3 - 12.0]	2 [1.7 - 2.6]	
CO (L/min)	4.3 (3.3 - 5.7)	5.1 (4.9 - 5.8)	
CI (L/min/m^2)	2.3 [1.8 - 2.9]	2.4 [2.2 - 2.9]	
Functional class (1/2/3/4) [%]	0 [0%] / 12 [17%] / 54 [76%] / 5 [7%]	0 [0%] / 5 [56%] / 4 [44%] / 0 [0%]	

Table 1. Baseline characteristics of subjects.

mPAP – mean pulmonary artery pressure, PAWP – pulmonary artery wedge pressure, CO – cardiac output, CI – cardiac index. Data presented as median (IQR) unless stated.

*Patients on bridging therapy with phosphodiesterase 5 inhibitors whilst waiting for

pulmonary endarterectomy surgery.

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	CTEPH Pre PEA	CTEPH Post PEA	p	p corrected
RAP (mmHg)	**8 [6 - 11]**	**6 [5 - 9]**	**0.019**	**0.028**
mPAP (mmHg)	**48 [38 - 53]**	**25 [22 - 33]**	**<0.001**	**<0.001**
PVR (WU)	**8.5 [4.8 - 13.0]**	**2.7 [2.2 - 4.0]**	**<0.001**	**<0.001**
CI (L/min/m^2)	2.3 [1.8 - 3.1]	2.5 [2.2 - 2.8]	0.511	0.511
6mwt distance (m)	**250 [195 - 362]**	**363 [295 - 408]**	**<0.001**	**<0.001**
FC (1/2/3/4) [%]	**0 [0%] / 8 [17%] / 37 [78%] / 2 [4%]**	**15 [32%] / 25 [53%] / 7 [15%] / 0 [0%]**	**<0.001**	**<0.001**
IL6 (pg/ml)	0.1 [0.1 - 4.5]	0.1 [0.1 - 4.1]	0.313	0.334
IL8 (pg/ml)	**7.4 [2.2 - 14.3]**	**4.7 [0.1 - 8.5]**	**0.017**	**0.027**
IL10 (pg/ml)	**1.6 [0.3 - 3.6]**	**1.1 [0.3 - 2.9]**	**0.015**	**0.027**
TNFα (pg/ml)	9.3 [5.7 - 17.1]	7.9 [4.2 - 14.5]	0.047	0.058
VEGFa (pg/ml)	**136.1 [14.7 - 366.9]**	**144.8 [13.7 - 289.6]**	**0.011**	**0.022**
VEGFc (pg/ml)	**35.3 [6.9 - 62.8]**	**17.1 [6.9 - 41.1]**	**<0.001**	**<0.001**
VEGFd (pg/ml)	**72.6 [18 - 121.3]**	**31.7 [6.9 - 69.3]**	**0.001**	**0.002**
Ang2 (pg/ml)	**1679.7 [570.4 – 2963.9]**	**753.2 [430.4 – 1234.5]**	**<0.001**	**<0.001**
BMP9 (pg/ml)	20.0 [9.8 – 37.8]	23.7 [7.2 – 45.8]	0.137	0.157
Endoglin (pg/ml)	**167.7 [34.4 – 411.8]**	**106.8 [27.4 – 258.9]**	**0.029**	**0.039**

Table 2. Changes in clinical assessments and serum proteins in CTEPH patients following PEA (n = 47

in both pre and post-PEA groups) assessed by the Wilcoxon signed-rank test.

RAP – right atrial pressure, mPAP – mean pulmonary artery pressure, PVR – pulmonary vascular resistance, CI – cardiac index, IL – interleukin, TNFα – tumour necrosis factor α, VEGF – vascular endothelial growth factor, Ang2 – angiopoietin 2, BMP9 – bone morphogenetic protein 9. **Bold** – p corrected < 0.05 (false discovery rate)

396

	Hazard ratio (95% confidence interval)	**p**
hsCRP	1.040 [1.000 - 1.080]	0.047
Age	1.052 [0.984 - 1.125]	0.138
6mwt distance	0.994 [0.988 - 1.001]	0.087

Table 3. Multivariable Cox proportional hazards model showing that hsCRP is an independent prognostic marker. n = 140.

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403 **FIGURES**

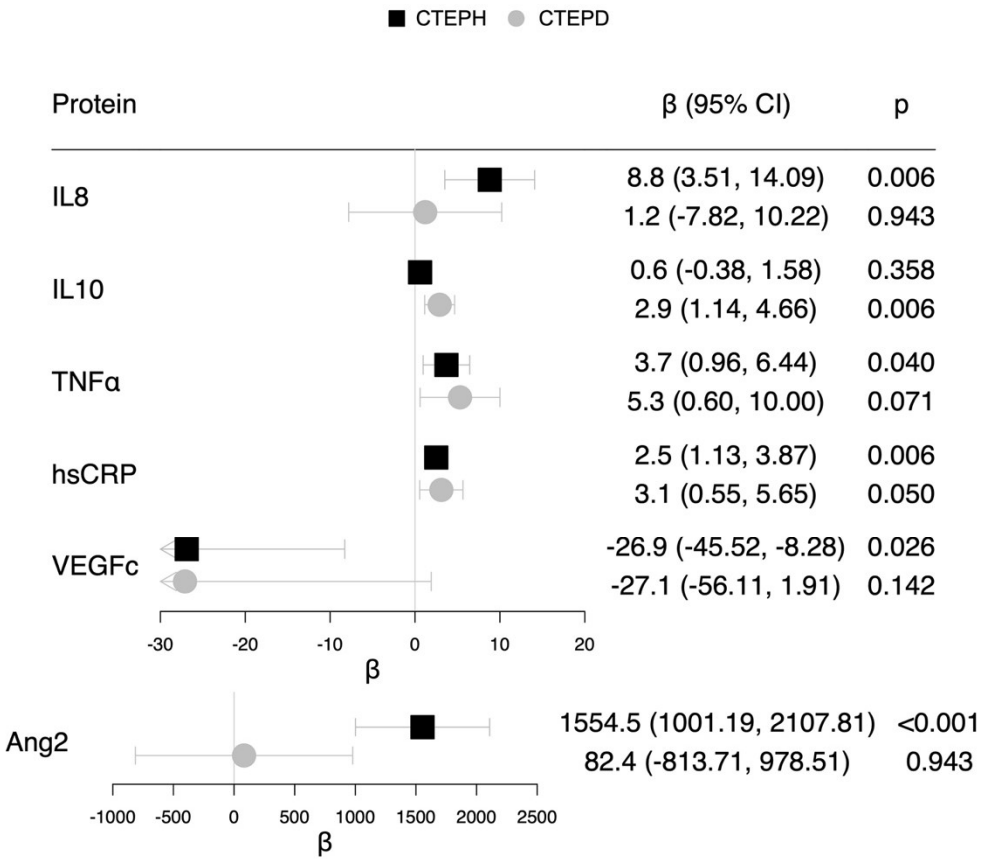

Figure 1. Forest plots showing proteins significantly different to controls (n = 20) in CTEPH (n = 71) and CTEPD (n = 9) patients pre-PEA in rank regression models including age, gender and batch as covariates.
β = estimated regression coefficient from rank regression model (positive value indicates increase in disease groups compared to controls). p = false discovery rate adjusted p value from rank regression models.

404

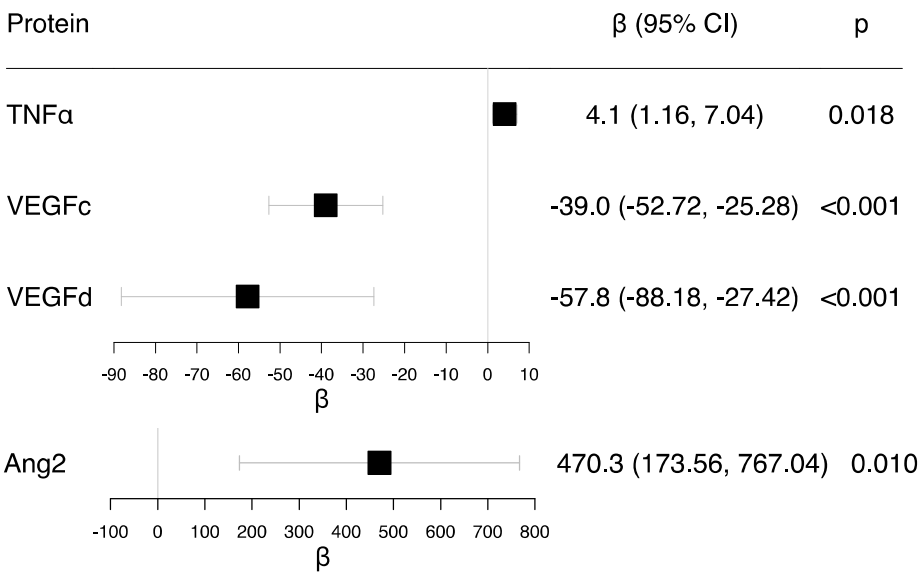

Figure 2. Forest plot showing significantly different serum proteins in CTEPH patients post-PEA (n = 47) compared to controls (n = 20) in rank regression models (using sex, gender and batch as covariates).
β = estimated regression coefficient from rank regression model (positive value indicates increase in CTEPH post-PEA group compared to controls). p = false discovery rate adjusted p value.

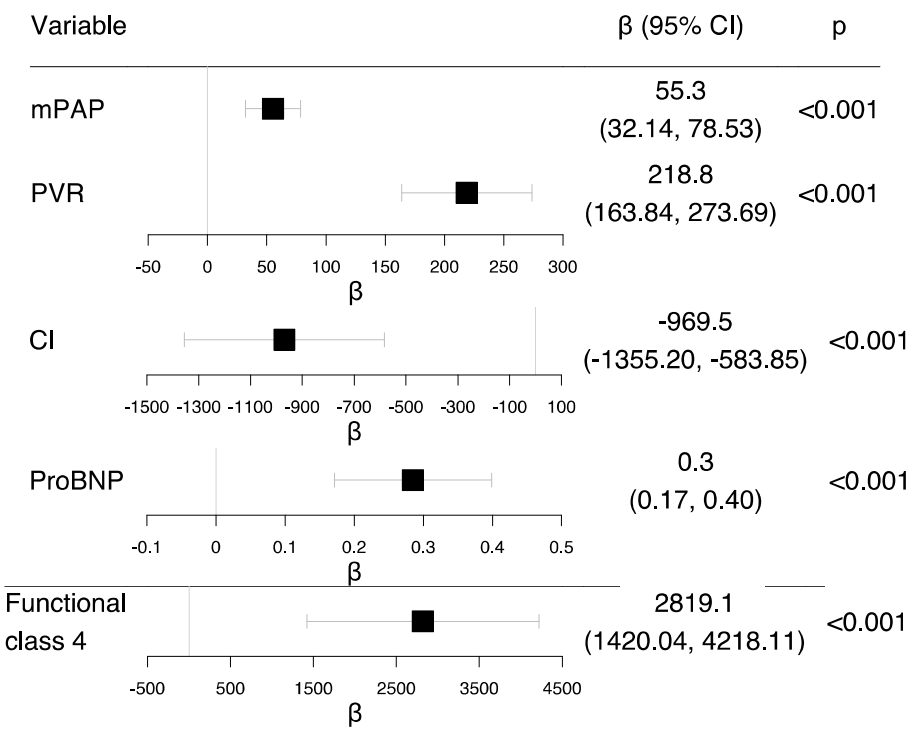

Figure 3. Forest plot showing significant associations between preoperative measurements of Ang2 and clinical assessments.
β = estimated regression coefficient from rank regression model. p = false discovery rate adjusted p value.

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