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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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.

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

76 **INTRODUCTION**

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

88

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

96

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

102

103 **METHODS**

104 Study design

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

112

113 Study subjects

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

116 occlusions without pulmonary hypertension (CTEPD; mPAP < 25 mmHg, n = 9) were included
117 as a disease comparator, because their disease is thromboembolic in origin but they have not
118 developed pulmonary hypertension at rest. Twenty age and sex matched volunteers were
119 recruited for comparison. A further 20 incident patients (not on pulmonary artery vasodilator
120 therapies) with IPAH were included as an additional disease comparator. The validation
121 dataset included 277 CTEPH patients and 26 CTEPD patients without PH, who had serum
122 samples biobanked pre-PEA in our Tissue Bank.
123
124 Serum collection and biomarker measurement
125 CTEPH and CTEPD patients had serum samples collected from a peripheral vein pre-PEA
126 (either at the time of initial right heart catheterisation or on admission prior to PEA [for
127 patients referred for surgery from other UK National Pulmonary Hypertension Centres]). In a
128 subset of patients, further sampling was performed at the first follow up visit to Papworth
129 Hospital 3-6 months post-PEA. This time point minimizes perioperative changes that may also
130 influence circulating protein levels. Healthy volunteer control and IPAH samples were
131 processed according to the same protocol.
132
133 Systemic levels of cytokines and angiogenic proteins were measured in duplicate wells using
134 either a customized human cytokine/chemokine magnetic bead assay (IL6, IL8, IL10, TNFα) or
135 a customized human angiogenesis/growth factor assay (angiopoietin 2 [Ang2], endoglin,

136 vascular endothelial growth factor [VEGF] a, VEGFc, VEGFd and bone morphogenetic protein
137 9 [BMP9]; Millipore, UK). Pre-PEA and post-PEA samples for each patient were placed on
138 adjacent wells on the same plates. high-sensitivity C-Reactive Protein (hsCRP) and Ang2 were
139 measured in a clinical pathology laboratory in pre-PEA samples. hsCRP was measured using
140 latex enhanced immunoturbidimetric assay on the Siemens Dimension EXL Autoanalyser.
141 Ang2 was measured using the MesoScale Discovery electrochemiluminescence immunoassay
142 on specially formatted microtitre plates.
143
144 In the validation dataset, hsCRP and Ang2 were measured in the same clinical pathology
145 laboratory using the platforms described above.
146
147 Statistical analysis
148 Statistical analyses were performed using R (www.r-project.org). Rank based regressions
149 were used to analyse the data [22]. These regression coefficients can be interpreted in the same
150 way as in a linear model. Cox-proportional hazards models were used to assess survival.
151 Please see the online supplement for more details.
152
153 **Results**
154 Subject characteristics
155 The baseline characteristics of patients recruited to the discovery arm are presented in Table

156 1 and in the online supplement.

157

158 Angiopoietin 2 is elevated in CTEPH compared to healthy controls

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

167

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

174

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

176 differences were seen in Ang2 levels between CTEPH patients and either CTEPD patients or
177 IPAH patients (Supplementary Table 3). CTEPH patients had lower VEGFd levels compared to
178 IPAH ($p_{corrected} < 0.05$).
179
180 Given there were only 9 patients with CTEPD without PH in the discovery dataset, a further
181 comparison between CTEPH and CTEPD was made in the validation dataset (see below).
182
183 Ang2 levels fell significantly following PEA surgery
184 Changes to circulating serum proteins following PEA surgery were assessed. Serum protein
185 levels were measured in 47 CTEPH patients pre- and post-PEA. Significant improvements in
186 haemodynamic and functional assessments were observed post-PEA (Table 2). Significant
187 reductions in Ang2, IL8, IL10, VEGFc, VEGFd, and endoglin were observed (Table 2, $p_{corrected} <$
188 0.05). There was a significant increase in VEGFa ($p_{corrected} = 0.02$).
189
190 In comparison with controls, the CTEPH post-PEA group had significantly higher Ang2 and
191 TNFα levels ($p_{corrected} < 0.05$; Figure 2), as well as significantly reduced VEGFc and VEGFd levels
192 ($p_{corrected} < 0.001$).
193
194 Ang2 was consistently associated with pulmonary haemodynamic measurements and
195 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>

216 Ang2 was measured in a further 303 patients (277 with CTEPH and 26 patients with CTEPD
217 without PH) to validate the key findings described above and further investigate the
218 significance of these biomarkers in CTEPH. PEA was performed in 72% of CTEPH patients and
219 27% of CTEPD patients. The clinical characteristics of these patients are provided in
220 Supplementary Table 4.
221
222 hsCRP was also assessed as a measure of inflammation and to validate the association
223 between hsCRP and mortality reported by Skoro-Sajer et al [23]. hsCRP and Ang2 were strongly
224 associated in a multivariable model including age, sex and PVR as covariables (estimated
225 regression coefficient = 0.17, standard error = 0.03, $p < 0.001$).
226
227 Ang2 and hsCRP levels were significantly higher in CTEPH patients compared to controls in
228 rank regression models using age and gender as covariables (Supplementary Table 5).
229 Importantly, Ang2 was significantly lower in patients with CTEPD without PH compared to
230 CTEPH patients ($p < 0.001$). Median (IQR) values for Ang2 were: control 4094 pg/ml (3457 -
231 5180), CTEPD 4554 pg/ml (3408 - 5575) and CTEPH 8197 pg/ml (4995 - 16851).
232
233 Significant associations between pre-PEA Ang2 and pre-PEA mPAP, PVR, CI and 6mwt distance
234 were once again demonstrated in the validation dataset (Supplementary Table 6).
235 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**

256 In this study of serum biomarkers in CTEPH we investigated two pathobiological processes
257 previously associated with pulmonary hypertension and impaired thrombus resolution. We
258 show that Ang2 is increased in patients with CTEPH compared to both healthy controls and,
259 importantly, patients with CTEPD without pulmonary hypertension. Ang2 is associated with
260 pulmonary haemodynamics and falls following PEA, as recently revealed by Hobohm et al [20].
261 We show that hsCRP is an independent prognostic marker of survival in this patient group,
262 supporting data from Skoro-Sajer et al [23]. Our data also suggests other molecules such as
263 endoglin, IL10 and VEGF molecules may be differentially expressed in CTEPH compared to
264 controls and other disease states.
265
266 Hobohm et al. demonstrated that Ang2 plays a critical role in CTEPH pathogenesis; by
267 impairing thrombus resolution. In an extensive body of work, they describe high levels of
268 Ang2 in organising PEA specimens and show Ang2 impairs venous thrombus resolution in two
269 different models [20]. They suggest that Ang2 levels are higher in CTEPH patients compared to
270 IPAH patients. Previously, Kümpers et al. had shown Ang2 expression is increased in plexiform
271 lesions from patients with IPAH and found Ang2 to be an independent prognostic marker of
272 survival in this group [10].
273
274 Our data validates aspects of the study from Hobohm et al. and makes new observations that
275 extends our knowledge of Ang2 in CTEPH. The multivariable statistical analysis is a strength

276 of our study. We hypothesise that Ang2 is a marker of distal vasculopathy and/or endothelial
277 dysfunction. Supporting this Ang2 is highest in CTEPH patients, as they have the most
278 vasculopathy, and lowest in controls, as they have no vasculopathy. While patients with
279 CTEPD without PH, may have no discernible vasculopathy and therefore low circulating Ang2.
280
281 We observed Ang2 expression associated with endothelialized channels within the chronic
282 thromboembolic material (Supplementary Figure 2). Hobohm et al., have also shown
283 increased expression of Ang2 in PEA specimens. However, we show that patients with CTEPD
284 without PH had near normal circulating levels of Ang2, significantly lower than CTEPH
285 patients, despite the presence of proximal chronic thromboembolic material. This suggests
286 that the obstructive chronic thromboembolic material is not the *predominant* source of
287 circulating Ang2 in CTEPH. We suggest that the source of elevated circulating Ang2 is more
288 likely pulmonary endothelial dysfunction, analogous to previous studies in IPAH. We show
289 that, if PVR is considered, there is no difference in Ang2 between CTEPH and IPAH groups. We
290 cannot exclude the right ventricle as a source of Ang2 but to the best of our knowledge Ang2
291 is not expressed in cardiac myocytes. Our study and that of Hobohm et al., demonstrates the
292 importance of Ang2 in disease pathogenesis, both in the initiation of disease (impaired
293 thrombus resolution) and severity of disease (distal vasculopathy).
294
295 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

336 pathophysiology and tease out from which “compartment” differences may arise, as they do
337 not fulfil the haemodynamic criteria for PH and do not have clinically overt heart failure.
338 Although, we have previously demonstrated these patients do not have entirely normal
339 cardiac physiology [34]. This group of patients have not been well characterized, but with the
340 growing recognition of a spectrum of disease referred to as post PE syndrome this is an
341 important avenue for future research [35,36].
342
343 The study has several limitations. There are limited numbers of patients with CTEPD. Much
344 larger numbers overall are needed to assess the potential interactions with confounders such
345 pulmonary artery vasodilator therapies and different forms of anticoagulation. We have not
346 assessed Ang2 expression in areas of vasculopathy to prove this is a source of circulating Ang2.
347 However, we believe the vasculopathy in CTEPH is similar to that seen in IPAH. Kümpers et al.
348 showed that Ang2 was upregulated in areas of vasculopathy [10]. Experimental models of CTEPH
349 do not fully recapitulate human disease; therefore large, well-controlled observational
350 studies are required to further our understanding of disease pathogenesis. The study has
351 identified several interesting signals, such as differences in VEGF molecules and IL10 between
352 patients with CTEPD and CTEPH. Further studies are required to validate them and assess
353 their significance.
354
355 *Summary*

356 By targeted screening of key proteins related to inflammation, vascular homeostasis and
357 angiogenesis, we provide useful insights into CTEPH pathobiology. IL8, IL10, TNFα and VEGF
358 molecules are altered in CTEPH and may play a part in disease pathogenesis. Circulating levels
359 of Ang2 are associated with pulmonary haemodynamics and Ang2 may be a marker of distal
360 pulmonary circulation remodelling. Ang2 may have a role in assessing future treatments that
361 affect distal pulmonary artery remodelling/vasculopathy in CTEPH and other forms of PH.
362 Finally, we demonstrate that hsCRP is an independent prognostic marker in patients with
363 CTEPH undergoing PEA. This study adds to an increasing body of evidence that the interplay
364 between inflammatory cell signalling (a key trigger event in many forms of PH) and the
365 subsequent angiogenic response to insult may play a key role in disease development.
366

367 **DISCLOSURES**

368 None

369

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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.

395

	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.

397

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399

400

401

402

403 **FIGURES**

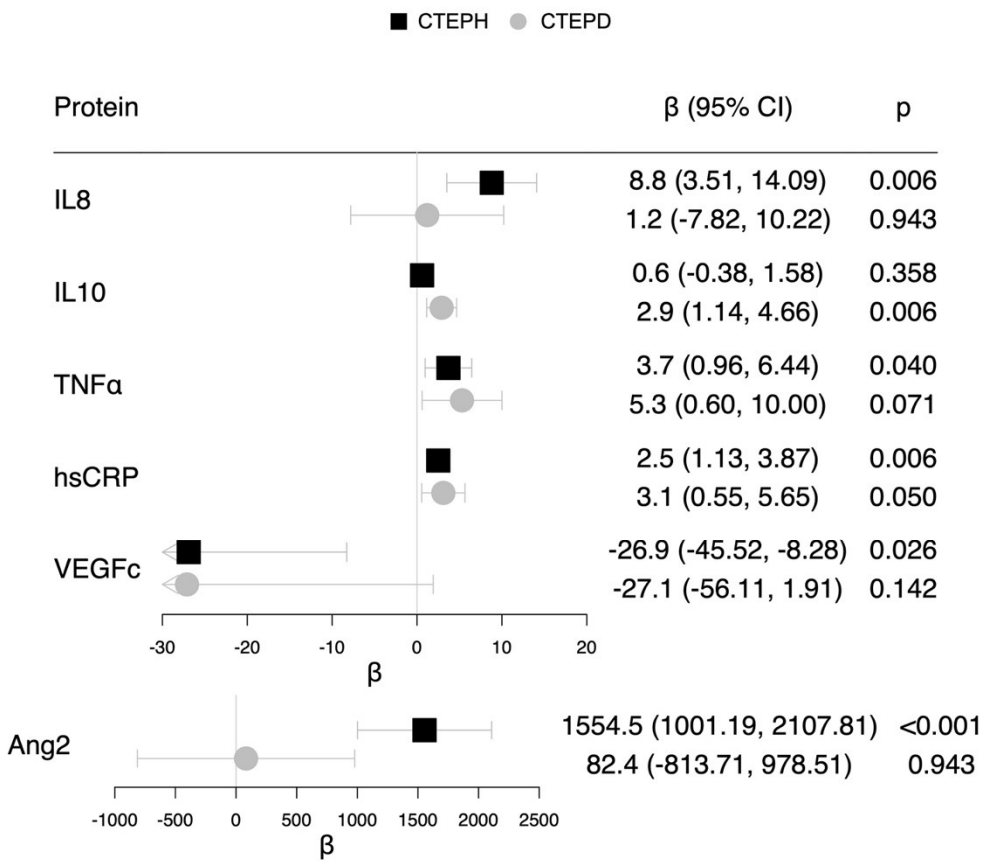

Figure 1. Forest plots showing proteins significantly different to controls (n = 20) in CTEPH (n = 71) and CTEPD (n = 9) patients pre-PEA in rank regression models including age, gender and batch as covariates.
β = estimated regression coefficient from rank regression model (positive value indicates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

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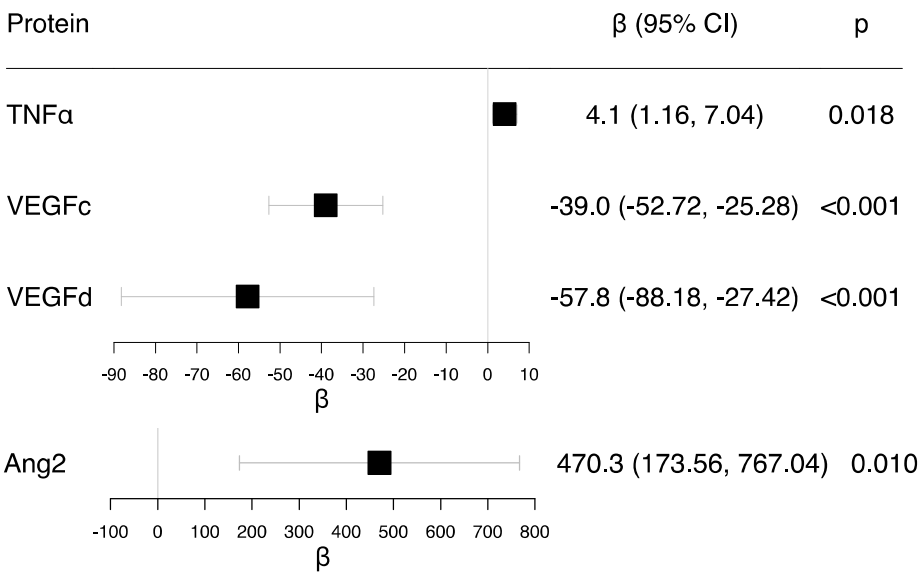

Figure 2. Forest plot showing significantly different serum proteins in CTEPH patients post-PEA (n = 47) compared to controls (n = 20) in rank regression models (using sex, gender and batch as covariates).
β = estimated regression coefficient from rank regression model (positive value indicates increase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

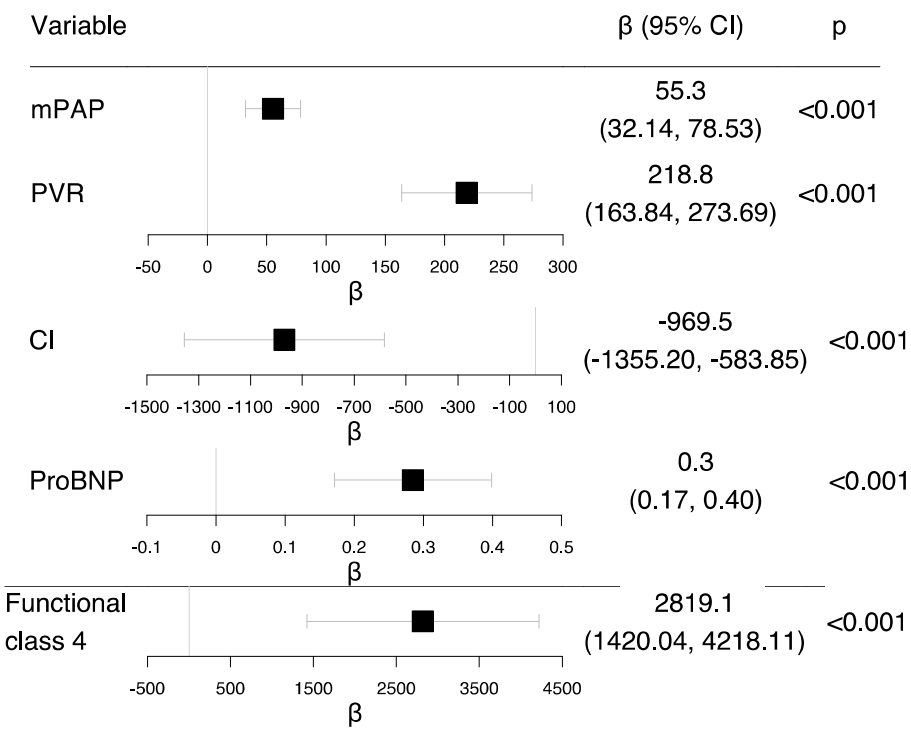

Figure 3. Forest plot showing significant associations between preoperative measurements of Ang2 and clinical assessments.
β = estimated regression coefficient from rank regression model. p = false discovery rate adjusted p value.

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