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Proinflammatory cytokine levels are linked with death in pulmonary arterial hypertension

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Short sentence of 130 characters or less summarising the most important findings, or message:

Proinflammatory cytokines are independently associated with mortality in patients with pulmonary arterial hypertension.

Recent data suggest that inflammatory processes play a major role in pulmonary vascular remodeling in patients with idiopathic, heritable, and drug or toxin-induced pulmonary arterial hypertension (ihdPAH) [1, 2]. Besides gaining insight into the mechanisms of PAH, understanding the link between inflammation and PAH may help to identify future therapeutic targets. Different studies have shown increased levels of cytokines in ihdPAH, among which are the proinflammatory cytokines IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12p70, tumour necrosis factor–α, MCP-1 [2, 3], and the cytokine-like hormone leptin [4]. Advances in highly sensitive multiplex detection technologies present new opportunities to rapidly and specifically measure many cytokines using a limited sample volume [5]; while the potential of combining markers rather than focusing on any single biomarker is of considerable interest [3, 6]. Using such an approach, Soon et al recently suggested that IL-2, IL-6, IL-8, IL-10 and IL-12p70 levels were associated with mortality in patients with ihdPAH. The French Network of Pulmonary Hypertension recently conducted a multicentre prospective cohort study of incident cases of PAH followed-up during 3 years [7], from which a biobank of plasma stored as microstraws at -180°C was available. Therefore, we tested our prospective cohort as to whether cytokines quantified using a highly sensitive multiplex assay may predict mortality in patients with ihdPAH.

Briefly, consecutive incident cases of PAH presenting at the university pulmonary vascular departments in Clamart (Paris), Marseille, Grenoble, Strasbourg, Nancy, and Toulouse, were enrolled between December 2003 and April 2006. Incident cases were defined as PAH patients with a diagnosis of PAH confirmed by right-heart catheterization, without current or previous treatment with drugs approved for the management of PAH such as endothelin receptor antagonists, type 5 phosphodiesterase inhibitors or prostacyclins.

For each patient, 24 mL venous blood samples taken during initial diagnostic right heart catheterization using the central venous line were anonymized and immediately

transferred to the Annemasse French Blood Service biobank, where EDTA plasma and serum microstraws were stored at -180°C. In February 2011, EDTA plasma microstraws, available from 74 patients with ihdPAH were sent via an express carrier in dry ice to Grenoble University Hospital, France for cytokine quantification. Cytokines were quantified using Randox® multiplex assays (Antrim, UK), using cytokine arrays I and V. The cytokines quantified are listed in table 1. Death, and cause of death were verified from hospital records, contact with primary care physicians, and when required using the Inserm French Mortality Registry. The primary study outcome was all-cause mortality.

Patients were censored at 3 years or at time of lung or heart/lung transplantation. We used univariable Cox proportional hazards models to analyse the unadjusted association between baseline characteristics and all-cause mortality. We estimated the adjusted hazard ratios (HR) of death and their corresponding 95% confidence intervals (CI) using a multivariable Cox proportional hazards model. P-values of less than 0.05 were considered statistically significant. All analyses were performed using Stata version 11 (Stata Corporation, College Station, TX, USA).

The analysed sample consisted of 74 patients with ihdPAH. The mean age for all patients was 51.5 (standard deviation [SD], 17.5) and 38 (51.3%) were male. Baseline patient characteristics have been previously reported [7] and plasma cytokine levels are presented in Table 1.

Seventeen patients died within 3 years of enrolment. In univariable analysis, the baseline characteristics associated with increased hazard of death included NYHA class IV (HR 17, 95% CI 1.75-164, P= 0.01) and lower values of the six-minute walk distance (HR 0.41, 95% CI 0.24-0.69 for 1 standard deviation increment, P= 0.001) while a non-significant trend towards greater hazard of death was observed for age (HR, 1.69, 95% CI 0.97-2.94, P=

0.06) and right atrial pressure (HR 1.56, 95% CI 0.94-2.59, P = 0.08). The proinflammatory cytokines IL-1α, IL-1β, IL-6, TNF-alpha and IL-13 were associated with increased unadjusted hazard of death (Table 1). After adjusting for age, six minute walk distance, cardiac output, and right atrial pressure, higher levels of proinflammatory cytokines IL-1α, IL-1β, TNF-alpha and IL-13 remained independently associated with mortality while IL-6 was associated with a non-significant trend towards increased hazard of death (Table 1).

We therefore confirm previous reports showing that the levels of various proinflammatory cytokines can predict mortality in a homogenous group of patients with ihdPAH. While we observed differences in terms of cytokine prognosis, IL-6 consistently predicts poor outcome in the three available studies[8, 9].

It remains unclear how inflammation contributes to the pathogenesis of PAH. It is likely that cytokines are produced as a result of an initial activation of adaptive immunity to propagate further the inflammatory process on their own or through the production of growth factors[2]. However, we did not observe any association between growth factor levels and mortality. The observed link between cytokine levels and mortality does not imply that the blockade of a specific cytokine receptor will improve patient outcome. Indeed, the whole range of inflammatory cytokines seems to be activated, and further physiopathological investigation is required to determine whether one specific pathway may be prominent. Furthermore, this link provides a rationale for targeting inflammation, as recently performed in animal models where dexamethasone reversed monocrotaline-induced pulmonary hypertension in rats [10]. Such an approach could be tested using IL-6 receptor blockers such as tocilizumab.

The main limitation of our study relates to the interval between plasma sampling and cytokine quantification. When considering cytokine measurement, several issues should be considered in order to maintain cytokine stability: firstly samples should be stored at low

temperatures (at least -80°C); secondly multiple freezing thawing cycles should be avoided; thirdly, while most cytokines remain stable within a 2-year period, longer term storage may alter cytokine levels as a result of cross reactivity between protein epitopes [11]. Despite the fact that samples were kept at -180°C and that they did not undergo any cycle of freezing-thawing as used samples do not re-enter the biobank, the storage time was long, from 5 to 8 years. Therefore, we cannot exclude cytokine degradation and we could not test it given that routine multiplex analysis was unavailable at study conception. This may explain why our levels of IL-6, 10, 12, 13 and TNF-α were lower than those of Soon et al[8]. A second potential limitation is the high male/female ratio. The most plausible explanation is that it may be due to chance in a small cohort. The second potential explanation is that that being female is a strong positive prognostic factor [12], implying that cohorts enrolling incident cases are more likely to enrol men than cohorts based on prevalent cases.

In conclusion, our data further support the concept that proinflammatory cytokines are independently associated with mortality in patients with PAH, emphasize the possible role of inflammation in PAH, and support the interest of targeting inflammation when developing future PAH therapeutic strategies. The current routine availability of multiplex assays may also prompt proinflammatory cytokine quantification in order to help refine patients' prognosis at the time of diagnostic right heart catheterization.

Table 1. Baseline plasma cytokine levels and associated hazard ratios for death for patients with idiopathic, familial, and drug-induced PAH (n =74).

Cytokine	Mean level, pg/mL (SD)		Unadjusted Hazard Ratios (95% CI)*		P	Adjusted Hazard Ratios (95% CI)†		P
IL-1α	0.52	(1.11)	1.35	(1.01-1.82)	0.04	1.74	(1.25-2.43)	0.001
IL-1β	2.82	(3.89)	1.41	(1.01-1.97)	0.04	1.43	(1.01-2.03)	0.04
IL-2	1.88	(4.03)	1.31	(0.92-1.84)	0.13	-	-	-
IL-3	4.07	(5.75)	1.42	(0.89-2.26)	0.14	-	-	-
IL-4	1.24	(1.38)	1.04	(0.65-1.67)	0.87	-	-	-
IL-6	3.64	(5.21)	1.45	(1.09-1.92)	0.01	1.33	(0.98-1.81)	0.06
IL-7	4.01	(3.32)	0.75	(0.41-1.38)	0.36	-	-	-
IL-8	35.7	(92.4)	1.07	(0.72-1.60)	0.73	-	-	-
IL-10	1.65	(10.67)	0.71	(0.12-4.24)	0.71	-	-	-
IL-12p70	2.64	(3.90)	0.96	(0.59-1.57)	0.89	-	-	-
IL-13	1.28	1.93	1.54	(1.00-2.38)	0.05	1.81	(1.08-3.01)	0.02
IL-23	5	6	1.44‡	(0.57-3.66)	0.44	-	-	-
IFNγ	0.72	(1.10)	1.09	(0.68-1.76)	0.72	-	-	-
TNFα	3.07	(1.68)	1.50	(1.14-1.96)	0.004	1.69	(1.14-2.51)	0.009
MCP1	138	(70)	1.18	(0.83-1.68)	0.35	-	-	-
VEGF	61.9	(76.2)	0.85	(0.48-1.50)	0.57	-	-	-
EGF	19.5	(30.7)	0.75	(0.35-1.60)	0.45	-	-	-

CI: confidence interval, PAH: pulmonary arterial hypertension, SD: standard deviation.
*Hazard ratios correspond to 1 standard deviation increment in the values except for IL23, for which the HR corresponds to the detection of values higher than 0.1 ng/mL.
†Hazard ratios were adjusted for age, six minute walk distance, cardiac output, and right atrial pressure. Missing values for covariates were imputed by means of multiple imputations.

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Conflict of interest:

JLC declares receiving unrestricted research grants from Pfizer, Boiron, and Actelion. This also concerns CC as a member of the same household. FC declares receiving research grants and lecture fees from drug companies including Actelion, Bayer-Shering, GSK, Pfizer and United Therapeutics. OS has relationships with drug companies including Actelion, BayerSchering, GSK, Lilly, Pfizer and United Therapeutics; in addition to being investigator in trials involving these companies, relationships include consultancy services and membership of scientific advisory boards. GS and MH have relationships with drug companies including Actelion, BayerSchering, GSK, Lilly, Novartis, Pfizer and United Therapeutics; in addition to being investigators in trials involving these companies, relationships include consultancy services and membership of scientific advisory boards. BD, JL,CS, AC, MRG, PF, and AY declare they have no conflicts of interest to disclose.

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