Plasma Interleukin-6 Adds Prognostic Information in Pulmonary Arterial Hypertension

Gustavo A. Heresi, MD, Metin Aytekin, PhD, Jeffrey P. Hammel, MS, Sihe Wang, PhD, Soumya Chatterjee, MD, and Raed A. Dweik, MD

1Department of Pulmonary and Critical Care Medicine, Respiratory Institute, Cleveland Clinic, Cleveland, Ohio; 2Department of Medical Biology, Faculty of Medicine, and Genome & Stem Cell Center, Erciyes University, Kayseri, Turkey; 3Department of Clinical Pathology, Pathology and Laboratory Medicine Institute; 4Department of Rheumatologic and Immunologic Disease, Orthopedic and Rheumatologic Institute; Cleveland Clinic, Cleveland, Ohio

Correspondence to: Raed A. Dweik, MD, 9500 Euclid Avenue A90, Cleveland, OH 44195; Tel (216) 445-5763, Fax (216) 445-8160, E-mail: dweikr@ccf.org
To the Editor:

Non-invasive biomarkers are needed to aid in making challenging clinical decisions in pulmonary arterial hypertension (PAH). Several biomarkers have been described, but only the natriuretic peptides have gained clinical utility. A key question to answer is whether or not a new biomarker adds independent incremental information [1]. Novel PAH markers may be discovered from new pathobiologic pathways, such as inflammation. Interleukin 6 (IL-6) has been particularly linked with the development of severe pulmonary hypertension in animal models, mimicking the pathology of human disease [2]. IL-6 is a major regulator of the production of C-reactive protein (CRP), a marker of cardiovascular risk [3]. We conducted this study to determine if these inflammatory biomarkers add incremental prognostic information in PAH.

This is a cohort study based on a prospective Biobank. We enrolled patients with idiopathic, heritable, connective tissue-, and congenital heart disease-associated PAH, as defined by current guidelines [4], between March 2005 and June 2011. The study was approved by the Cleveland Clinic Institutional Review Board. We used enzyme linked immunosorbent assay (R&D catalog number D6050) to measure IL-6, the Abbott platform for highly-sensitive CRP (hsCRP), and chemiluminiscence immunoassay for B-type natriuretic peptide (BNP). Peripheral plasma samples were kept at −80 °C until retrieved for the measurement of the biomarkers (September 2011 for hsCRP and BNP, and February 2012 for IL-6). Investigators who performed the biomarker determination were blinded to the study’s participants’ outcomes.

All-cause mortality since the date of study blood sampling was ascertained via manual and automated query of the electronic medical record, as well as query of the Social Security Death Index. Lung transplantation was censored. The same day as study blood sampling, the following
data were collected: New York Heart Association (NYHA) functional classification, six-minute walk distance (6MWD), echocardiography, and percent predicted diffusion capacity for carbon monoxide (DLCO%). Right heart catheterization data obtained closest to the date of study blood sampling were recorded (median time 27 days, interquartile range 5–66 days). Analyses were performed using JMP® Pro 9.0.0 and R version 2.14.2. We used receiver operating characteristic curves (ROC) analysis, Kaplan Meier curves and Cox models to determine the associations between biomarkers and mortality. We also used recursive partitioning tree-based analysis using the R function ‘rpart’ to identify the group of biomarkers and baseline variables that had optimal discriminatory ability with regards to all-cause mortality. Each possible threshold of biomarker and baseline variable was evaluated to find the split that formed two groups with the greatest difference in survival (alive versus dead). Survival was measured by estimated event rates after exponential scaling, which accounts for the time each subject was followed. Within the patient subsets defined by the first split, the process was repeated to identify optimal splits at a second level. This repeated splitting was stopped when goodness-of-fit of the tree model could no longer be improved with further splits, or when resulting subgroups fell in size to less than 20 patients.

We enrolled 76 patients, 62 female, with a mean age of 51 ± 14 years. The types of PAH included 40 idiopathic, 10 heritable, 19 connective tissue disease (13 scleroderma) and 7 congenital heart disease. The cohort had a mean 6MWD of 360 ± 127 meters, mean PAP 51 ± 13 mmHg, right atrial pressure (RAP) 10 ± 6 mmHg, cardiac index 2.4 ± 0.8 L/min/m² and pulmonary vascular resistance 11 ± 7 Wood units. Sixty-three patients were on PAH-targeted therapies, 32 of them on parenteral prostacyclin.
The median (interquartile range) concentrations of the biomarkers were: IL-6 3.82 (1.79 – 9.87) pg/mL, hsCRP 5.7 (1.95 – 14.85) mg/L, and BNP 59 (19 - 132) pg/mL. Plasma IL-6 correlated with the 6MWD (r = 0.52, p < 0.0001) and RAP (r = 0.26, p = 0.03). HsCRP correlated with the 6MWD (r = 0.40, p = 0.001). During a median follow up 2.8 years (interquartile range 1.6 – 4.6 years), there were 22 deaths and 5 lung transplants. Both IL-6 and hsCRP were univariate predictors of mortality. On ROC curve analysis, IL-6 had better discriminant ability (IL-6 area under the curve 0.81, hsCRP area under the curve 0.68). IL-6 concentrations ≥ 4.7 pg/mL had a sensitivity of 86% and a specificity of 72%, and were associated with 9-fold increase in the risk of death (Figure, panel A). An hsCRP level ≥7.5 mg/L was less strongly associated with mortality (HR 4.80, 95% CI 1.77 – 13.1, p< 0.001, sensitivity 77%, specificity 67%). Other univariate predictors of mortality included male sex, NYHA class, 6MWD, BNP, pericardial effusion, RAP and red cell distribution width. In a multivariable Cox model, male sex, BNP and IL-6 were independently associated with mortality. When IL-6 was removed from the model given its collinearity with hsCRP (r = 0.71), then hsCRP entered the model. In the decision tree model however, only BNP, IL-6 and cardiac index were independent predictors.

In our cohort we confirmed that BNP levels above 180 pg/mL, as previously reported [5, 6], were associated with an extremely poor prognosis, with 3-year survival of only 16% (HR for death 13.01, 95% CI 4.98 – 35.19, p <0.0001). Thus, we were interested in the prognostic utility of IL-6 and hsCRP in patients with BNP levels below 180 pg/mL (n = 60). In this sub-population, IL-6 ≥ 4.7 pg/mL, RAP and DLCO% were the only independent predictors of mortality in a forward stepwise Cox model (all p < 0.05), while BNP was not. Recursive partitioning tree-based analysis showed that the combination of 6MWD (cutoff 450 meters), RAP (cutoff 8 mmHg)and IL-6 (cutoff 5.6 pg/mL) offered the highest ability to separate groups of patients with significantly
different survival. In this model, BNP was not found to provide any predictive information either.

Panel B in the figure shows the survival curves for the tree-identified groups. The best prognosis was seen in patients with good 6MWD or low RAP (group 1). Patients with more limited walk distance and an elevated RAP, but with low plasma IL-6 levels had an intermediate prognosis (group 2). Those with limited 6MWD, high RAP and elevated plasma IL-6 had the worse outcome (group 3) (see Figure, panel B).

Plasma IL-6 levels were independent predictors of survival, both in a multivariable Cox proportional hazards model and in a recursive partitioning tree-based analysis. Importantly, plasma IL-6 provided prognostic information in conjunction with 6MWD and RAP in patients with BNP levels below the established cut point of 180 pg/mL [5, 6]. It is very clear that patients with elevated BNP levels have a poor prognosis, However, our study shows that below this threshold, BNP does not provide any prognostic information. In this subset, plasma IL-6 in conjunction with 6MWD and RAP allow for further risk stratification. HsCRP on the other hand, did not add independent information in PAH. As 69 patients had blood drawn more than 2 years prior to IL-6 measurement, uncertainty about IL-6 stability is a study limitation. However, our data is consistent with previous IL-6 findings [7-10]. Another limitation is the lack of a validation cohort and the modest sample size, which also did not allow us to enter other biomarkers into the models.

In conclusion, plasma IL-6 provides incremental prognostic information in PAH, especially in patients with low BNP levels.

REFERENCES


**FIGURE LEGEND**

**Figure.** **Panel A.** Transplant-free survival according to a interleukin-6 (IL-6) receiver operating characteristic curve identified cutoff of 4.7 pg/mL. Hazard ratio 9.26, 95% CI 2.74 – 31.3, p < 0.0001. **Panel B.** Transplant-free survival of 60 PAH patients with B-type natriuretic peptide (BNP) levels < 180 pg/mL according to recursive partitioning tree-based analysis identified groups. Group 1 (n = 37): 6-minute walk distance (6MWD) ≥ 450 meters or 6MWD < 450 meters and right atrial pressure < 8 mmHg; 3-year survival 100%. Group 2 (n = 14): 6MWD < 450 meters and RAP ≥ 8 mmHg and IL-6 < 5.6 pg/mL; 3-year survival 78%. Group 3 (n = 9): 6MWD < 450 meters and RAP ≥ 8 mmHg and IL-6 ≥ 5.6 pg/mL; 3-year survival 42%.