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Circulating Collagen Biomarkers as Indicators of Disease Severity in Pulmonary Arterial Hypertension

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ABSTRACT

OBJECTIVES The goal of this study was to determine if biomarkers of collagen metabolism in PAH identify patients with worse disease and higher risk of death.

BACKGROUND The relationship between circulating markers of collagen metabolism, degree of disease severity, and outcome in pulmonary arterial hypertension (PAH) is unknown.

METHODS Patients with stable idiopathic, anorexigen-associated, and hereditary PAH were prospectively enrolled. Levels of the following collagen biomarkers were measured: N-terminal pro-peptide of type III procollagen (PIIINP), C-terminal telopeptide of collagen type I (CITP), matrix metalloproteinase (MMP)-9, and tissue inhibitor of metalloproteinase (TIMP)-1. Patients were divided into mild, moderate, and severe PAH groups. Data were compared between tertiles of each biomarker. Pearson correlation and Spearman rank coefficient analyses were performed. Data on time to death or transplantation were examined by Kaplan-Meier survival curves.

RESULTS Circulating levels of PIIINP, CITP, MMP-9, and TIMP-1 were higher in the PAH group ($n = 68$) as compared with age- and sex-matched healthy controls ($n = 37$) ($p < 0.001$ for each). PIIINP levels increased with the severity of disease ($p = 0.002$). PIIINP tertile data indicated that with increasing levels, 6-min walk distance and cardiac index decreased, World Health Organization functional classification worsened, and resting heart rate increased. A significant correlation existed between PIIINP levels and worsening World Health Organization functional classification ($r_s = 0.320$; $p < 0.01$), and there was a negative correlation between cardiac index and 6-min walk distance ($r = -0.304$ and $r = -0.362$, respectively; $p < 0.05$). PIIINP tertiles showed a trend toward worse outcome in patients with higher tertiles (lung transplant or death) ($p = 0.07$; log-rank test).

CONCLUSIONS Markers of collagen metabolism were associated with worse disease in patients with PAH. (J Am Coll Cardiol HF 2014;2:412-21) © 2014 by the American College of Cardiology Foundation.

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Pulmonary arterial hypertension (PAH) is a terminal disease characterized by pulmonary vascular remodeling resulting in right heart failure and death. Vascular remodeling and fibrosis are among the key pathological features in PAH. One of the main features of vascular remodeling seen in PAH is collagen deposition in the remodeled pulmonary vessels. The best way to quantify collagen deposition in the pulmonary vasculature is by tissue analysis at autopsy or of explanted lungs. Antemortem assessment of collagen in the pulmonary vasculature is not possible with current imaging, and lung biopsy is not considered safe.

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Type I and III collagen, the most abundant forms of collagen in the human lungs, provide architectural support for the alveolar walls, vessels, visceral pleura, and tracheobronchial tree and are primarily synthesized and secreted by lung fibroblasts as procollagen precursor molecules with pro-peptides at both ends (1). The N-terminal pro-peptide of type III procollagen (PIIINP) is used as a biological marker of collagen metabolism because it is not completely removed from its procollagen precursor (2). Carboxy-terminal telopeptide of type I collagen (CITP) is a marker of extracellular collagen I degradation. Historically, matrix metalloproteinase (MMP)-9, a gelatinase that degrades most fibrillar collagen, is considered a marker of extracellular matrix breakdown. However, recent data suggest that MMP-9 may play an important role in the inflammatory response and control of angiogenesis (3–6). Tissue inhibitor of metalloproteinase (TIMP)-1 is a ubiquitous inhibitor of all MMPs.

Collagen production and smooth muscle cell proliferation occurs in small pulmonary arteries of patients with severe PAH (7,8). Studies have shown that the transpulmonary gradient of procollagen III occurs in healthy subjects undergoing cardiac catheterization, suggesting that normal human lungs can actively synthesize collagen (9). It has been established that elevated procollagen III levels in the serum mirror changes in bronchoalveolar lavage of patients with sarcoidosis (10), interstitial pulmonary fibrosis (11), *Pneumocystis carinii* pneumonia (12), acute lung injury (13), and acute respiratory distress syndrome (14,15), indicating that such parenchymal changes are reflected in peripheral blood samples. These studies suggest that ongoing collagen metabolism in the pulmonary vascular bed can be assessed by measuring circulating levels of collagen metabolites. Accordingly, the objectives of this study were to investigate the relationship between circulating markers of collagen metabolism, degree of disease severity, and outcome in a well-characterized PAH cohort.

METHODS

SUBJECTS. After obtaining institutional review board approval and written informed consent, consecutive subjects with PAH and age- and sex-matched healthy controls who met inclusion/exclusion criteria were prospectively enrolled in a cross-sectional observational study. Patients with PAH were enrolled from the new and established patient population followed at the Pulmonary Hypertension Clinic at Baylor College of Medicine. The diagnosis of PAH was established by the presence of mean pulmonary artery pressure ≥25 mm Hg and pulmonary capillary wedge pressure ≤15 mm Hg. The mean time between right heart catheterization and study enrollment was 1.3 ± 1.6 years. Inclusion criteria for patients with PAH were age of 18 years or older, ability to provide written informed consent, and stable PAH therapy for 1 month before enrollment. Detailed inclusion/exclusion criteria for the patients with PAH and controls are outlined in the Online Appendix. Patients with PAH were followed for up to 61 months (2.8 ± 1.4 years; range: 1.2 to 5.1 years) after enrollment, and data on transplant-free survival, transplantation, and death were collected. Histologically, the available lungs and heart tissue of 2 patients with PAH who underwent transplantation were examined. Hematoxylin and eosin stain was used to outline the pulmonary vascular remodeling, and trichrome stain was used to document collagen staining. To define the severity of PAH, patients were divided into mild, moderate, and severe PAH groups. The mild PAH group was defined by a 6-min walk distance (6MWD) of >440 m, World Health Organization functional classification (WHO FC) I to II, and right atrial pressure (RAP) ≤10 mm Hg. The severe PAH group was defined by a 6MWD of ≤350 m, WHO FC III to IV, and RAP ≥15 mm Hg. The moderate PAH group fell between the criteria for mild and severe PAH (16).

BIOCHEMICAL MEASUREMENTS OF INDICES OF COLLAGEN METABOLISM. Blood was drawn once from a peripheral vein for biomarker measurements, transferred immediately into a glass tube, and allowed to clot. Serum was separated from the

ABBREVIATIONS AND ACRONYMS

6MWD = 6-min walk distance

BNP = brain natriuretic peptide

CITP = carboxy-terminal telopeptide of type I collagen

MMP = matrix metalloproteinase

PAH = pulmonary arterial hypertension

PIIINP = N-terminal pro-peptide of type III procollagen

RAP = right atrial pressure

RV = right ventricular

TIMP = tissue inhibitor of metalloproteinase

WHO FC = World Health Organization functional classification