CHEST

Elevation of Plasma Cell-Free Hemoglobin in Pulmonary Arterial Hypertension

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BACKGROUND: Cell-free hemoglobin (CFH) is a potent nitric oxide scavenger associated with poor outcomes in several diseases. Pulmonary arterial hypertension (PAH) is characterized by reduced nitric oxide availability. We hypothesized that CFH would be elevated in PAH and would associate with hemodynamics and clinical outcomes.

METHODS: We measured CFH in 200 consecutively evaluated patients with PAH, 16 unaffected bone morphogenetic receptor protein type 2 (BMPR2) mutation carriers, 19 healthy subjects, and 29 patients with pulmonary venous hypertension (PVH). CFH values were tested for association with hemodynamics, time to hospitalization, and death.

RESULTS: CFH was elevated in patients with PAH and BMPR2 carriers compared with healthy subjects and patients with PVH ($P \leq .01$ all comparisons). There were no differences in CFH across PAH subtypes. CFH modestly correlated with mean pulmonary artery pressure ($\rho = 0.16$, $P = .03$) and pulmonary vascular resistance ($\rho = 0.21$, $P = .01$) and inversely with cardiac index ($\rho = -0.18$, $P = .02$) in patients with PAH. CFH was not associated with hemodynamic response to nitric oxide or death. Patients with the highest CFH levels had increased risk of PAH-related hospitalization when adjusted for age, sex, and PAH cause (hazard ratio, 1.69; 95% CI ,1.08-2.66; $P = .02$).

CONCLUSIONS: CFH is elevated in patients with PAH and BMPR2 carriers compared with healthy subjects and patients with PVH. Elevated CFH levels are independently associated with an increased risk of hospitalization. Further study is required to understand the mechanism of CFH elevation and the potential pathologic contribution of CFH in PAH.

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ABBREVIATIONS: BMPR2 = bone morphogenetic protein receptor type II; CFH = cell-free hemoglobin; CTD-PAH = connective tissue disease-associated pulmonary arterial hypertension; HPAH = heritable pulmonary arterial hypertension; IPAH = idiopathic pulmonary arterial hypertension; IQR = interquartile range; MCV = mean corpuscular volume; mPAP = mean pulmonary artery pressure; PAH = pulmonary arterial hypertension; PAP = pulmonary artery pressure; PVH = pulmonary venous hypertension; PVR = pulmonary vascular resistance; PWP = pulmonary wedge pressure; RDW = RBC distribution width; RHC = right-sided heart catheterization; UMC = unaffected mutation carrier

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Hemoglobin, when released from the RBC, is a potent oxidant[1,2] and vasoconstrictor[3-7] associated with poor clinical outcomes.[8] Cell-free hemoglobin (CFH) levels are elevated in the plasma of patients with sickle cell anemia,[3,9] sepsis,[8] and after RBC transfusion.[5] In all of these patient populations, CFH has been associated with poor outcomes, including the risk of acute kidney injury,[1] myocardial infarction,[10] and death.[8] Potential mechanisms underlying this association include the ability of CFH to injure the vascular endothelium,[6,11] cause oxidative injury,[1] and scavenge nitric oxide,[3] all of which lead to vasoconstriction.

Pulmonary arterial hypertension (PAH) is characterized, in part, by vasoconstriction of the pulmonary vascular bed.[12] Activation of the nitric oxide signaling pathway is a major therapeutic avenue in PAH.[13-15] In an animal model of hypoxia-induced PAH, infusion of cell-free hemoglobin in mice was associated with increased pulmonary artery pressure (PAP) and right ventricular size.[16] In humans with PAH, abnormalities in proteins responsible for hemoglobin processing have been reported.[17,18] There are no reports of CFH measurement in the general PAH population or any association with hemodynamics or clinical outcomes. Therapies directed toward preventing the negative effects of CFH are currently being developed[19]; these therapies could also be studied in patients with PAH if CFH is found to be associated with poor clinical outcomes.

We used a prospective institutional registry and biorepository to test the hypothesis that CFH would be elevated in PAH compared with healthy subjects and patients with pulmonary venous hypertension (PVH), in whom elevated pulmonary pressures are not related to nitric oxide imbalance. We also compared CFH levels in carriers of a mutation associated with heritable PAH, bone morphogenetic protein receptor type 2 (BMPR2), who did not have PAH at the time of enrollment. We further hypothesized that CFH levels would be associated with severity of pulmonary vascular disease and clinical outcomes. The purpose of this study was to determine whether a link exists between elevation in CFH, a potent nitric oxide scavenger, and PAH, a disease characterized by decreased nitric oxide availability.

Materials and Methods

Study Populations

The Vanderbilt University Institutional Review Board approved this study, and all patients gave written informed consent (Vanderbilt University IRB numbers 9401 and 111530). Subjects with PAH for this study were consecutively enrolled in the Vanderbilt Pulmonary Hypertension Research Cohort, a prospective institutional registry containing detailed clinical information and biologic specimens collected over 30 years.[20] We identified 200 consecutive patients with PAH presenting for their initial evaluation in the Vanderbilt Pulmonary Vascular Clinic between 2007 and 2012. The Vanderbilt Pulmonary Hypertension Research Cohort also includes unaffected mutation carriers (UMCs) of a BMPR2 mutation. Patients with PAH were diagnosed by experienced clinicians according to consensus guidelines.[21] PAH was defined as an invasively measured mean pulmonary artery pressure (mPAP) $\geq$ 25 mm Hg as well as a pulmonary wedge pressure (PWP) or left ventricular end-diastolic pressure $\leq$ 15 mm Hg. PAH patient inclusion was restricted to patients with idiopathic PAH (IPAH), heritable PAH (HPAH), or portopulmonary PAH and PAH associated with congenital heart disease and connective tissue disease.

Additionally, we studied three control populations: healthy subjects without known cardiopulmonary disease, patients with PVH, and UMCs. UMCs were studied to determine whether genetic predisposition to PAH is associated with elevated CFH as a potential marker of subclinical pulmonary vascular disease or a mediator of pulmonary vascular disease. We selected consecutive patients with PVH due to ischemic or nonischemic cardiomyopathy enrolled between 2010 and 2012 in the Vanderbilt Main Heart Registry, which includes clinical information and plasma samples from consenting patients evaluated at the Vanderbilt Heart and Vascular Institute. Inclusion in this group required mPAP $>$ 25 mm Hg and PWP $>$ 15 mm Hg measured on right-sided heart catheterization (RHC) closest to the date of blood draw. All patients with PVH had a transpulmonary gradient (mPAP minus PWP) $\leq$ 18 mm Hg, indicating the absence of a significant vasoreactive contribution to pulmonary hypertension.[22]

RHC was performed with a balloon-tipped catheter using hemodynamic and fluoroscopic guidance. Heart rate, right atrial pressure, PAP, PWP, and cardiac output were recorded from the RHC closest to the date of blood draw. Cardiac index, pulmonary vascular resistance, and stroke volume were calculated from standard formulas. In patients with PAH, acute vasodilator testing was performed using inhaled nitric oxide, as described previously by our group.[23]

Blood Collection and Analysis

Samples were obtained at the time of clinic visits or hospitalization for patients, and via the Vanderbilt General Clinical Research Center for healthy control subjects and UMCs. Patient samples were not specifically obtained prior to the initiation of therapy or RHC. Plasma samples were collected using standard blood sampling techniques into ethylenediaminetetraacetic acid plasma tubes. Plasma was collected either from central venous access catheters or via venipuncture. Ethylenediaminetetraacetic tubes were centrifuged within 45 min at 4,000 rpm and the plasma fraction immediately aliquoted as 200-μL aliquots and stored at -80°C. CFH was measured in duplicate in human plasma using spectrophotometric methods with the QuantiChrom Hemoglobin Assay Kit (BioAssay Systems).

Statistical Analysis

All values are reported as mean $\pm$ SD or median (interquartile range [IQR]) and categorical variables as absolute value and percent. Between-group differences were calculated using Mann-Whitney *U* test or χ^2 test. Correlation coefficients were calculated using the Spearman method. The impact of CFH on hemodynamics was assessed using linear regression with adjustment for age, sex, and PAH cause. Time to event analysis was performed using the Kaplan-Meier log-rank test and Cox regression to assess the impact of CFH on time to first hospitalization or death. We prespecified a Cox regression analysis controlling for PAH cause, age, and sex, as these variables are known to influence survival and clinical course. Statistical analyses were performed using SPSS 20 software (IBM).