Hepatoma-derived Growth Factor Predicts Disease Severity and Survival in Pulmonary Arterial Hypertension

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Abstract

Rationale: Pulmonary arterial hypertension (PAH) is a fatal disease, and pulmonary microvascular remodeling is an important contributor to PAH development. Therefore, we hypothesized that a circulating angiogenic factor could predict disease severity and survival.

Objectives: We sought to assess the relationship of serum hepatoma-derived growth factor (HDGF) with PAH disease severity and survival.

Methods: Using a newly developed enzyme-linked immunosorbent assay, we evaluated circulating HDGF levels in two independent PAH cohorts and two different characterized control cohorts. Clinical and laboratory data were also used to assess the value of HDGF as a PAH prognostic biomarker.

Measurements and Main Results: Serum HDGF levels were significantly elevated in two independent PAH cohorts. Importantly, serum HDGF levels were not elevated in a noncardiac chronic disease

cohort. Further, patients with elevated HDGF had significantly lower exercise tolerance, worse New York Heart Association functional class, and higher levels of N-terminal pro-brain natriuretic peptide. HDGF was a strong predictor of mortality, with an unadjusted hazard ratio of 4.5 (95% confidence interval, 1.9–10.3; $P = 0.003$ by log-rank test). In multivariable Cox proportional hazards models, elevated HDGF levels predicted decreased survival after being adjusted for age, PAH subtype, invasive hemodynamics, and N-terminal pro-brain natriuretic peptide.

Conclusions: Elevated HDGF was associated with worse functional class, exertional intolerance, and increased mortality in PAH, suggesting HDGF as a potential biomarker for predicting mortality and as having possible diagnostic value for distinguishing PAH from non-PAH. HDGF may add additional value in PAH risk stratification in clinical trials and may represent a potential target for future PAH drug development.

Keywords: pulmonary arterial hypertension; hepatoma-derived growth factor; biomarker; enzyme-linked immunosorbent assay; survival

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At a Glance Commentary

Scientific Knowledge on the

Subject: Pulmonary arterial hypertension (PAH) is a fatal disease, and current therapeutic monitoring and prognostic markers have many limitations.

What This Study Adds to the

Field: We discovered that hepatoma-derived growth factor can significantly predict mortality in PAH, with possible diagnostic value for distinguishing PAH from non-PAH.

Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by sustained elevation of pulmonary vascular resistance (PVR). The inciting events leading to a cascade of pathological changes that characterize PAH are poorly understood (1, 2). Although therapeutic options for pulmonary hypertension continue to expand, treatment is often begun late in the disease course, and, in that setting, clinicians use treatment aimed at advanced symptoms rather than using abrogative therapy aimed at the inciting events, curative therapy, or specific therapy tailored to heterogeneous PAH subtypes.

Given that treatment is not curative, but rather aimed at palliation of pathobiological processes, detection and monitoring of PAH disease progression and treatment response are of paramount importance. Unfortunately, invasive monitoring methods, such as hemodynamics (3, 4), and functional measures, such as 6-minute-walk distance (6MWD) (5, 6), can be inadequate predictors. Similarly, blood-based assays are useful clinically, but they have limitations for therapeutic monitoring and prognostication. N-terminal pro-brain natriuretic peptide (NTproBNP), which is currently the most commonly used pulmonary hypertension biomarker, is confounded by higher levels in children than in adults (7) and also by systemic disease. NTproBNP is a cardiac biomarker of chamber distention and therefore is used as a general diagnostic standard for heart failure with poor discrimination of right or left ventricular etiology (8, 9). Cardiac catheterization, the invasive method, can be

complicated by accruing morbidity and mortality risk, particularly in pediatric patients (10–12).

For all the above reasons, a stable and more lung- and/or vascular system-specific, blood-based biomarker could improve noninvasive prognostication and risk stratification, as well as monitoring of therapeutic efficacy. Hepatoma-derived growth factor (HDGF) is a secreted multifunctional protein that is highly expressed in pulmonary endothelial cells as an angiogenic factor (13–17). It is also involved in lung remodeling by stimulating bronchial and alveolar epithelial cell growth (18). HDGF requires nuclear translocation for mitogenic activity (19, 20), but it also functions in a poorly understood paracrine manner when secreted into culture media (21, 22). As pulmonary vascular remodeling is one of the signature events in PAH pathogenesis (23), HDGF could be involved in these dynamic changes in the pulmonary microvasculature.

In this study, we developed an ELISA to measure circulating serum HDGF concentrations and evaluated the potential value of HDGF as a PAH diagnostic and prognostic indicator. Some of the results of this study have been reported previously in the form of an abstract (24).

Methods

Study Cohorts

All study cohorts were approved by the Johns Hopkins University Institutional Review Board, and informed consent was obtained from all subjects.

PAH Patient Cohorts

The Pulmonary Hypertension Breakthrough Initiative. The Pulmonary Hypertension Breakthrough Initiative (PHBI), a highly phenotyped cohort of patients with severe PAH, was funded by the Cardiovascular Medical Research and Education Fund. Blood samples were obtained from PHBI enrollees before lung transplant ($n = 39$; 56% idiopathic pulmonary arterial hypertension [IPAH], 44% pulmonary arterial hypertension associated with congenital heart disease [APAH-CHD]). See Tables 1 and 2, as well as the METHODS section in the online supplement, for further information.

The Johns Hopkins Pulmonary Hypertension Program. The Johns Hopkins Pulmonary Hypertension (JHPH) cohort was an independent cohort of adult patients seen at the JHPH Program ($n = 73$; 49% IPAH, 51% pulmonary arterial hypertension associated with connective tissue disease [APAH-CTD]) (Tables 1 and 2). The inclusion criteria, exclusion criteria, and clinical assessments and therapy were published previously (25). Data were collected prospectively from the JHPH Program, which maintains a registry. Patients 18 years of age or older who were diagnosed with PAH by right heart catheterization and evaluated between January 1, 2007, and December 31, 2012, were included and classified into etiological groups on the basis of current guidelines (26). PAH was defined as a mean pulmonary arterial pressure (mPA) greater than or equal to 25 mm Hg, pulmonary capillary wedge pressure less than or equal to 15 mm Hg, and PVR greater than or equal to 3 Wood units (27). The diagnosis of CTD was based on meeting one of the following definitions (28): the American College of Rheumatology criteria, the presence of at least three of five features of the CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), or definite Raynaud's phenomenon and the presence of a specific systemic sclerosis-related autoantibody. This cohort was used for validation, clinical correlations, and survival analysis.

Control Cohorts

Johns Hopkins healthy control cohort. Healthy adult volunteers supplied control serum ($n = 39$). These samples were matched for age, sex, and race to the PHBI cases (Table 2).

Chronic disease control cohort. Serum samples ($n = 66$) were obtained from an ongoing, prospective cohort of Johns Hopkins Emergency Department patients initially evaluated for suspected acute coronary syndrome. Patients who presented to the adult emergency department with chest pain and acute coronary syndrome were ruled out and discharged to home; however, these patients had multiple medical comorbidities. Patients with the following comorbidities were excluded from analysis: alcoholism, HIV, and congestive heart

Table 1. Demographics and Characteristics of Pulmonary Arterial Hypertension Cohorts

	PHBI Cohort	JHPH Cohort
n	39	73
Age, yr, median (IQR)	44 (20–62)	62 (38–79)
Female sex, n (%)	34 (87%)	58 (80%)
Race, n (%)		
EA	31 (80%)	61 (83%)
AA	2 (5%)	10 (13.6%)
Other	6 (15%)	2 (0.3%)
PAH subgroup, n (%)		
IPAH	22 (56%)	36 (49%)
APAH-CHD	17 (44%)	NA
APAH-CTD	NA	37 (51%)
6MWD, m, median (range)	298 (85–1,016)	383 (211–671)
NYHA functional class, n (%)		
I/II	0 (0%)	41 (56%)
III/IV	19 (49%)	32 (44%)
Unknown	20 (51%)	0 (0%)
Laboratory chemistry, median (range)		
BNP, pg/ml	767 (127–3,154)*	NA
NTproBNP, pg/ml	NA	2,089 (20–11,698)
HDGF, ng/ml	1.93 (0.06–20.2)	0.815 (0.09–21.8)
Hemodynamics, median (range)		
RAP, mm Hg	13 (3–22)	7 (2–17)
mPAP, mm Hg	54 (48–66)	42 (25–72)
PCWP, mm Hg	12 (10–13)	10 (5–15)
PVR, WU	11 (4–17)	10 (5–16)
CO, L/min	4.5 (2.2–9.7)	4.3 (2.6–8.1)
Cardiac index, L/min/m ²	2.1 (1.3–4.9)	2.4 (1.6–4.1)

Definition of abbreviations: 6MWD = 6-minute-walk distance; AA = African American; APAH-CHD = pulmonary arterial hypertension associated with congenital heart disease; APAH-CTD = pulmonary arterial hypertension associated with connective tissue disease; BNP = brain natriuretic peptide; CO = cardiac output; EA = European American; HDGF = hepatoma-derived growth factor; IPAH = idiopathic pulmonary arterial hypertension; IQR = interquartile range; JHPH = Johns Hopkins Pulmonary Hypertension; mPAP = mean pulmonary arterial pressure; NA = not available; NTproBNP = N-terminal pro-brain natriuretic peptide; NYHA = New York Heart Association; PAH = pulmonary arterial hypertension; PCWP = pulmonary capillary wedge pressure; PHBI = Pulmonary Hypertension Breakthrough Initiative; PVR = pulmonary vascular resistance; RAP = right atrial pressure; WU = Wood units.

*n = 12.

failure. Notable inclusions were diabetes, systemic hypertension, elevated cholesterol, obesity, and smoking. Demographic information is described in Table 2 and Table E1 in the online supplement.

Development of a Sandwich ELISA for HDGF

We developed a sandwich ELISA based on the Meso Scale Discovery assay platform (Meso Scale Discovery, Gaithersburg, MD).

Table 2. Demographics of All Cohorts

	PAH Cases		Control Subjects	
	JHPH	PHBI	JHH Control Subjects	Chronic Disease Control Subjects
N	73	39	39	66
Age, yr, median (IQR)	62 (38–79)	44 (20–62)	43 (26–65)	57 (40–70)
Female sex, n (%)	58 (80%)	34 (87%)	32 (82%)	38 (57%)
Race, n (%)				
EA	61 (84%)	31 (80%)	32 (82%)	21 (32%)
AA	10 (16%)	6 (15%)	7 (18%)	45 (68%)
Other	2 (0%)	2 (5%)	0 (0%)	0 (0%)

Definition of abbreviations: AA = African American; EA = European American; IQR = interquartile range; JHH = Johns Hopkins healthy; JHPH = Johns Hopkins Pulmonary Hypertension; PAH = pulmonary arterial hypertension; PHBI = Pulmonary Hypertension Breakthrough Initiative.

The HDGF-specific antibodies and calibrator were characterized previously (29–31). The detailed ELISA protocol is described in the METHODS section in the online supplement.

Statistical Analysis

Baseline characteristics are presented as median and interquartile range (IQR), number and percentage, or median and range, where appropriate and indicated. Histograms of serum HDGF in cases and control subjects demonstrated that HDGF was not normally distributed, and thus nonparametric analysis was used where appropriate. Correlation analyses were done with Spearman's coefficient. The chi-square test, Mann-Whitney *U* test, or Kruskal-Wallis test was used for comparisons between groups, where appropriate. To evaluate the performance of HDGF as a discriminator of the presence of PAH, the area under the curve (AUC) of the receiver operating characteristic (ROC) curve was calculated using values derived for the derivation cases (PHBI cases) and healthy control subjects. Kaplan-Meier survival curves were constructed and log-rank tests performed to compare survival distributions. The association between HDGF concentration and survival was also tested using Cox regression models. A *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed using Analyse-it Software (2009 version; Analyse-it Software, Leeds, UK) and MedCalc statistical software version 13.1.0 (2014 version; MedCalc Software, Ostend, Belgium).

Immunohistochemistry

For HDGF immunohistochemical localization of HDGF in the PAH lung, paraffin-embedded PHBI APAH-CHD peripheral lung tissue sections from four enrollees were immunostained with a rabbit polyclonal anti-HDGF antibody using a VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA) with Vector Red as the chromogen. Detailed immunohistochemical methods were described previously (14).

Results

Serum HDGF Levels in PAH

An HDGF sandwich ELISA was developed with the following metrics: a lower limit of detection of 0.029 ng/ml with an intraassay

coefficient of variation less than or equal to 4.51%, interassay coefficients of variation less than or equal to 9.7%, equivalent quantitation in serum and plasma, and stable with long-term storage at room temperature or 4°C for 7 days. See details in the RESULTS section and Figure E1.

Derivation cohort (PHBI cases). PHBI samples were obtained at a single time point (enrollment) from patients with severe PAH listed for lung transplant. The demographic information and clinical characteristics of the patients are summarized in Tables 1 and 2. Samples from 39 patients with PAH were available for analysis, their median age was 44 (IQR, 20–62) years at the time of enrollment, and 87% were female. Compared with age-, sex-, and race-matched Johns Hopkins healthy (JHH) control subjects ($n = 39$), circulating HDGF concentrations were significantly increased in PHBI PAH samples (median HDGF, 1.93 ng/ml vs. 0.29 ng/ml; $P < 0.0001$) in aggregate or in IPAH (1.44 ng/ml; $P < 0.0001$) and APAH-CHD (2.25 ng/ml; $P < 0.0001$) separately (Figure 1).

Validation cohort (JHPH cases). JHPH samples were obtained at a single time point (enrollment) and were used as an external validation cohort. Clinical characteristics of the JHPH cohort are summarized in Table 1. A total of 73 adult patients were available for analysis. The median age of patients at the time of enrollment was 62 (IQR, 38–79) years, and 58 (80%) of the patients were women. The median follow-up was 2.3 years (maximum, 7.2 yr). The overall mortality was 30%, with 22 deaths observed during the follow-up observational period.

As shown in Figure 2A, circulating median HDGF concentrations were significantly increased in aggregate (0.82 ng/ml) at enrollment as compared with healthy control subjects (0.29 ng/ml) ($P < 0.0001$). HDGF median concentrations were also significantly increased in the IPAH (0.63 ng/ml) and APAH-CTD (1.01 ng/ml) subgroups (Figure 2B) compared with healthy control subjects ($P = 0.0003$ and $P < 0.0001$, respectively).

HDGF Localization in the PAH Lung

To localize the vascular expression of HDGF in the PAH lung, we performed immunohistochemistry using the same rabbit polyclonal antibody used as a capture antibody in the ELISA. As shown in Figure 3, which shows a representative

Figure 1. Box-and-whisker plot of serum hepatoma-derived growth factor (HDGF) levels in Pulmonary Hypertension Breakthrough Initiative (PHBI) patients with pulmonary arterial hypertension ($n = 39$) (A) in aggregate or (B) in subgroups of idiopathic pulmonary arterial hypertension (IPAH) ($n = 22$) and pulmonary arterial hypertension associated with congenital heart disease (APAH-CHD) ($n = 17$) versus Johns Hopkins healthy (JHH) adult control (Ctrl) subjects ($n = 39$). Boxes represent the interquartile range, and the horizontal lines are the medians. Outliers are indicated with solid dots.

section from a PHBI APAH-CHD lung, HDGF was predominately and highly expressed in pulmonary vascular endothelial cells in a nuclear pattern, as previously shown for HDGF (14).

HDGF Levels Are Not Confounded by the Presence of Chronic Disease

To begin to understand the specificity of HDGF for PAH, we analyzed serum HDGF in a cohort of patients with existing chronic

Figure 2. Box-and-whisker plot of serum hepatoma-derived growth factor (HDGF) levels (A) in the Johns Hopkins Pulmonary Hypertension (JHPH) cohort with pulmonary arterial hypertension ($n = 73$) versus Johns Hopkins healthy (JHH) control (Ctrl) subjects ($n = 39$) and (B) in subgroups of pulmonary arterial hypertension associated with connective tissue disease (APAH-CTD) ($n = 37$) and idiopathic pulmonary arterial hypertension (IPAH) ($n = 36$) versus JHH Ctrl. Boxes represent the interquartile range, and the horizontal lines are the medians. Outliers are indicated with solid dots.

Figure 3. Hepatoma-derived growth factor (HDGF) localizes to pulmonary artery endothelial cells in the lung of a subject with pulmonary arterial hypertension associated with congenital heart disease. A section representative of Pulmonary Hypertension Breakthrough Initiative pulmonary arterial hypertension associated with congenital heart disease, immunostained for HDGF, is shown. Arrows indicate HDGF-positive arterial vascular endothelial cells lining the vascular lumen. Original magnification, $\times 40$.

non-heart failure conditions, including diabetes and/or systemic hypertension, elevated cholesterol, obesity, and smoking ($n = 66$; median age, 57 [IQR, 40–70] yr; 58% female). Demographic details are described in Table 2 and Table E1. As shown in Figure 4, median HDGF serum

Figure 4. Box-and-whisker plot of serum hepatoma-derived growth factor (HDGF) level in chronic disease cohort (median, 0.20 ng/ml; $n = 66$) versus Johns Hopkins healthy adult volunteers (JHH Ctrl) (median, 0.29 ng/ml; $n = 39$; $P = 0.072$). Boxes represent the 5–95% interquartile range, and the horizontal lines are the medians. Outliers are indicated with solid dots.

concentrations were not different between healthy and chronic disease control cohorts (0.29 ng/ml vs. 0.20 ng/ml, respectively; $P = 0.072$).

HDGF Correlates with Clinical Markers of PAH

We evaluated the relationship between serum HDGF and invasive resting hemodynamics, exercise tolerance assessed by 6MWD, and serum chemistry, including NTproBNP. Spearman's rank-order correlations were calculated because the serum HDGF data were not normally distributed. There was a weak but statistically significant negative correlation with 6MWD ($r_s = -0.31$; $P = 0.003$) in the cases in aggregate (PHBI and JHPH cohorts) (Table E2). There was also only a modest negative correlation of serum HDGF with hemodynamics, specifically right atrial pressure (RAP) ($r_s = -0.2$, $P < 0.05$) in the JHPH cohort only. There were no statistically significant relationships between serum HDGF and NTproBNP, serum sodium, or serum creatinine.

Serum HDGF Discriminates PAH from Control Subjects

As normal circulating concentrations of HDGF are unknown, HDGF values from our derivation cohort (PHBI) and JHH control cohort were used to generate an ROC curve. Serum HDGF had the ability to identify the presence of PAH with an AUC of 0.89 ($P < 0.0001$) (Figure 5). On the basis of this ROC curve analysis, we established a serum HDGF threshold value of 0.7 ng/ml to distinguish healthy from PAH. This threshold value had a sensitivity and specificity for PAH of 73% and 85%, respectively. This threshold value was then applied to a cohort composed of PAH cases (JHPH cohort) and control subjects without PAH, specifically chronic disease control subjects. In this validation analysis with a case prevalence of 52%, the test performed well for the capacity to discriminate PAH, with positive and negative predictive values of 76% and 89%, respectively. To further evaluate the test characteristics, interval likelihood ratios were analyzed for all cases (PHBI and JHPH; $n = 112$) and control subjects (healthy and chronic disease; $n = 122$). For HDGF values less than 0.5 ng/ml, the likelihood ratio for the presence of PAH was 0.3 (95% confidence interval [CI], 0.2–0.4) for the presence of PAH. In contrast, for values of 0.75 ng/ml

or greater, the likelihood ratio was 8.1 (95% CI, 4.2–15.5) (Figure E2).

HDGF and PAH Severity

Using the identified threshold derived from ROC analysis in the derivation cohort (PHBI) (0.7 ng/ml), the JHPH cohort was dichotomized into two subgroups: those with levels below or above this threshold, denoted as HDGF_{low} ($n = 32$ [44%]) and HDGF_{high} ($n = 41$ [56%]), respectively (Table 3). The two subgroups were similar in age (58 vs. 56 yr), sex (78% vs. 83% female), race (81% vs. 85% European American), and PAH subtype (69% vs. 49% IPAH). Patients within the HDGF_{high} group had a shorter 6MWD ($P = 0.04$) and were more frequently in New York Heart Association functional class (NYHA-FC) III/IV ($P = 0.02$), consistent with poor exertional tolerance and symptoms. Additionally, HDGF_{high} patients had a similar trend toward higher NTproBNP values. The two groups did not discernibly differ in terms of invasive hemodynamic parameters. Thus, patients with higher HDGF had worse functional status and diminished functional capacity.

Serum HDGF and Outcomes in PAH

In the validation cohort (JHPH), HDGF was significantly lower in survivors than in nonsurvivors (0.2 ng/ml vs. 1.4 ng/ml; $P = 0.004$). Kaplan–Meier curves were generated to assess the relationship between elevated HDGF levels and mortality. Elevated HDGF levels (HDGF_{high} subgroup) were associated with a significantly increased risk of death, with an unadjusted hazard ratio of 4.5 (95% CI, 1.9–10.3; $P = 0.003$ by log-rank test) (Figure 6).

Cox proportional hazards models were constructed to examine the relationship between HDGF and outcomes. A high HDGF level was a significant predictor of adverse outcomes, with an unadjusted hazard ratio of 4.5 (95% CI, 1.5–13.3; $P < 0.01$) (Table 4). Multivariable models were built with adjustment for demographics, variables previously linked to adverse outcomes (32–34), and variables linked to increased mortality in univariate analysis (Table 4). Univariate analysis demonstrated associations between mortality and age, race, PAH subtype (i.e., APAH-CTD vs. IPAH), serum creatinine, sodium, NTproBNP, 6MWD, NYHA-FC, and individual hemodynamic variables,

Figure 5. Receiver operating characteristic curve for serum hepatoma-derived growth factor as a predictor of pulmonary arterial hypertension for a total of 39 cases from the Pulmonary Hypertension Breakthrough Initiative and 39 control subjects from the Johns Hopkins healthy adult volunteer control cohort (area under the curve, 0.89; $n = 78$; $P < 0.0001$).

specifically RAP, mPA, PVR, cardiac output (CO), and cardiac index, consistent with published data in other PAH cohorts (32–34). The relationship between HDGF levels and outcomes persisted in multivariable Cox proportional hazards models. A high HDGF predicted death after adjustment for age, subtype of PAH (i.e., IPAH vs. APAH-CTD), invasive hemodynamic variables (RAP, mean pulmonary arterial pressure, PVR, CO, and cardiac index), and NTproBNP (Table 4 and Figure 7). The strength of association was attenuated when we adjusted for some clinical parameters, specifically NYHA-FC and 6MWD, suggesting that these variables either confounded the relationship or shared a common causal pathway between HDGF and outcomes.

Discussion

This study demonstrates that HDGF was significantly increased in two independent PAH cohorts and in all three PAH subtypes examined. HDGF was able to distinguish PAH from non-PAH with an AUC of 0.89. The likelihood ratio for the presence of PAH was 0.3 (95% CI, 0.2–0.4) for HDGF values less than 0.5 ng/ml, but it increased dramatically to 8.1 (95% CI, 4.2–15.5) for HDGF values of 0.75 ng/ml or greater. HDGF levels inversely correlated with 6MWD but not invasive hemodynamic variables. Patients with elevated HDGF

levels had more severe PAH, as demonstrated by statistically significant decreased 6MWD, higher NYHA-FC, and median survival of 2.9 years. HDGF was also able to discriminate survivors from nonsurvivors and was associated with an unadjusted increased risk of death of 4.5 (95% CI, 1.9–10.3; $P = 0.003$). The relationship between HDGF levels and mortality persisted in multivariable Cox proportional hazards models after we adjusted for age, PAH subtype, invasive hemodynamic variables (RAP, mPA, PVR, CO, and cardiac index), and NTproBNP. Thus, circulating HDGF may have prognostic utility in risk stratification of PAH that extends past current clinical measures.

PAH is a progressive, incurable disease characterized by an inexorable increase in pulmonary arterial resistance, sustained elevation of pulmonary arterial pressure, and eventual right heart failure and death (2, 23, 35). Although the pathobiology of PAH development is not completely

understood, formation of plexiform lesions and microvascular remodeling are the hallmarks of PAH (23, 35). Both phenomena are related to endothelial dysfunction, abnormal growth, and/or unbalanced injury repair mechanisms. Growth and angiogenesis factors play central roles in these dynamic changes of endothelium.

From a biological perspective, HDGF is an intriguing biologic candidate for pulmonary hypertension. HDGF was originally purified from the conditioned media of the human hepatoma cell line Huh-7 (21). In further study, researchers found HDGF also to be highly expressed in the lung, developing heart, and pulmonary microvascular endothelial cells (14, 36). HDGF is involved in lung remodeling by stimulating bronchial and alveolar epithelial cell growth (18). Overexpression of HDGF or stimulation by recombinant HDGF significantly promotes vascular endothelial and smooth muscle cell proliferation and migration, indicating both

Table 3. Clinical Characteristics as a Function of Serum Hepatoma-derived Growth Factor

	HDGF _{low} ($n = 32$)	HDGF _{high} ($n = 41$)	P Value
Demographics			
Age, yr, median (range)	58 (50–62)	56 (52–72)	0.06
Female sex, n (%)	25 (78%)	34 (83%)	0.80
Race, n (%)			0.70
EA	26 (81%)	36 (85%)	
AA	6 (19%)	5 (12%)	
Other	0 (0%)	0 (0%)	
IPAH, n (%)	20 (69%)	21 (49%)	0.08
APAH, n (%)	12 (31%)	21 (51%)	
6MWD, m, median (range)	410 (369–475)	357 (300–415)	0.04
NYHA-FC, n			
I/II	23	17	0.02
III/IV	9	24	
Laboratory chemistry, median (range)			
NTproBNP, pg/ml	341 (136–1,794)	894 (410–3,378)	0.03
Sodium, mEq/L	140 (137–141)	139 (138–140)	0.37
Creatinine, mg/dl	1.0 (0.8–1.1)	1 (0.8–1.3)	0.15
Hemodynamics, median (range)			
RAP, mm Hg	8 (6–13)	6 (4–12)	0.07
mPAP, mm Hg	44 (34–53)	42 (29–50)	0.22
PVR, WU	8.3 (6.6–12.3)	7.3 (4.7–12.9)	0.84
CO, L/min	4.6 (3.8–6.2)	4.2 (3.5–5.2)	0.07
Cardiac index, L/min/m ²	2.5 (2.2–3.2)	2.3 (1.9–2.9)	0.31

Definition of abbreviations: 6MWD = 6-minute-walk distance; AA = African American; APAH = associated pulmonary arterial hypertension; CO = cardiac output; EA = European American; HDGF_{high} = hepatoma-derived growth factor level above 0.7 ng/ml cutoff; HDGF_{low} = hepatoma-derived growth factor level below 0.7 ng/ml cutoff; IPAH = idiopathic pulmonary arterial hypertension; mPAP = mean pulmonary arterial pressure; NTproBNP = N-terminal pro-brain natriuretic peptide; NYHA-FC = New York Heart Association functional class; PVR = pulmonary vascular resistance; RAP = right atrial pressure; WU = Wood units. Data are expressed as median (range) or number (percent).

Number at risk								
Group: Low	32	29	24	23	21	21	17	12
Group: High	41	34	25	22	19	16	13	10

Figure 6. Kaplan-Meier survival curve for serum hepatoma-derived growth factor (HDGF) in pulmonary arterial hypertension. The curve represents survival analysis of the Johns Hopkins Pulmonary Hypertension cohort dichotomized by serum HDGF levels ($n = 73$; $P = 0.003$).

autocrine and paracrine functionality (16, 17, 19, 20). Although the mechanism underlying HDGF's mitogenic function remains unknown, researchers demonstrated that the HDGF N-terminal PWWP domain binds to DNA (31, 37) and

that DNA binding was required for mitogenic activity (19, 20), possibly through its *trans*-regulation function (38). We have demonstrated that HDGF is a phosphoprotein, with serine 103 phosphorylation being regulated by the cell cycle and required for its mitogenic activity (39). Although HDGF was discovered in cell culture conditional media, implying a secretory mechanism, this secretory pathway and the potential presence of an HDGF receptor remain unknown. However, mitogen-activated protein kinase and p38 pathways were shown to be activated by exogenous HDGF stimulation, indicating the presence of receptor-mediated signal transduction pathways for HDGF (40, 41). HDGF is reexpressed in smooth muscle cells *in vivo* during neointimal formation after injury, suggesting that HDGF plays additional roles in vascular remodeling (42). HDGF also functions as an angiogenic factor, with previous studies demonstrating that HDGF stimulates vascular formation both *in vitro* and *in vivo* by a direct effect on endothelial growth and/or by induction of VEGF expression (14, 15, 17).

Recent clinical studies revealed that HDGF is a prognostic marker of pathologic cell growth and indicates poor outcome in lung, pancreatic, esophageal, and liver cancer (43–48). HDGF has a demonstrable role in disordered cell growth in multiple pathological processes; similarly, PAH is a disease epitomized by disordered cell growth, lending biological feasibility to HDGF as a PAH biomarker. However,

whether HDGF is a mediator and/or a surrogate of PAH progression remains unclear. It is also clear from our previous work and based on immunohistochemical evidence from the Human Protein Atlas (<http://www.proteinatlas.org/ENSG00000143321-HDGF/tissue/lung#img>) that, while HDGF is expressed in the lung vascular endothelium, it is not endothelium or lung specific. HDGF is also highly expressed in epithelia of multiple tissues, including the intestine, skin, and bronchus, and nonepithelial organs, such as the cerebellum, suggesting that HDGF circulating levels could be confounded by diseases of these organs. Additional studies are needed to delineate non-PAH conditions in which circulating HDGF levels are elevated.

HDGF levels were significantly increased in multiple PAH subtypes (IPAH, APAH-CHD, APAH-CTD), and high HDGF levels were associated with PAH severity (decreased 6MWD, increased NYHA-FC) and mortality, but only weakly with cardiac hemodynamics, indicating that HDGF could be a measure of PAH severity that is distinct from heart failure alone. Specifically, elevated levels of HDGF were not associated with worse right heart hemodynamics. As mentioned above, this suggests that HDGF is not a biomarker of heart failure similar to NTproBNP. BNP or its biologically inactive N-terminal tail (NTproBNP) is produced and released predominately with atrial distention that occurs with left, right, or biventricular dysfunction. Clinically, BNP is the only circulating pulmonary hypertension biomarker available and has been an important prognostic factor for pulmonary hypertension survival (49). However, BNP as a pulmonary hypertension biomarker can be confounded by chronic conditions that are not pulmonary hypertension specific, such as renal failure, left heart failure, and lung disease (7, 9). Although it is unknown whether HDGF levels are altered in renal failure, there was no discernible relationship between serum HDGF and renal function, and, more important, the capacity of HDGF to predict outcomes persisted after adjusting for renal function. HDGF was a significant predictor of survival, even after adjustment for multiple factors, including PVR and NTproBNP, suggesting that HDGF provides further prognostic granularity

Table 4. Multivariable Cox Proportional Hazards Models

	HR (95% CI)
HDGF	4.5 (1.5–13.3)*
Model adjusted for	
Age	3.9 (1.3–12.0) [†]
AA race	4.6 (1.6–13.4)*
Male sex	4.3 (1.5–12.8)*
APAH	3.9 (1.3–11.5) [†]
Sodium, mEq/L	4.4 (1.5–13.2)*
Creatinine, mg/dl	4.6 (1.5–13.7)*
NTproBNP	4.4 (1.3–15.7) [†]
NYHA-FC, I/II vs. III/IV	2.5 (0.8–7.7) [‡]
6MWD, m	5.6 (0.7–45.3) [‡]
RAP, mm Hg	7.0 (2.2–22.4)*
mPAP, mm Hg	4.7 (1.6–13.9)*
PVR	4.0 (1.4–12.0) [†]
CO	4.8 (1.3–18.6) [†]
Cardiac index	5.1 (1.5–17.2) [†]

Definition of abbreviations: 6MWD = 6-minute-walk distance; AA = African American; APAH = associated pulmonary arterial hypertension; CI = confidence interval; CO = cardiac output; HDGF = hepatoma-derived growth factor; HR = hazard ratio; mPAP = mean pulmonary arterial pressure; NTproBNP = N-terminal pro-brain natriuretic peptide; NYHA-FC = New York Heart Association functional class; PVR = pulmonary vascular resistance; RAP = right atrial pressure.

* $P < 0.01$.

[†] $P < 0.05$.

[‡] $P \geq 0.05$.

Figure 7. Cox proportional hazards analysis of the Johns Hopkins Pulmonary Hypertension cohort adjusted for serum N-terminal pro-brain natriuretic peptide (NTproBNP), right atrial pressure (RAP), cardiac index (CI), and pulmonary vascular resistance (PVR) (hazard ratio [HR], 5.8; 95% confidence interval, 1.4–23.7; $P = 0.014$ by log-rank test). HDGF = hepatoma-derived growth factor.

than can be achieved with current clinical measures.

As demonstrated, HDGF is uniquely suited to be a PAH biomarker, given its ability to predict survival in PAH, equivalence to NTproBNP performance,

correlation with a decline in 6MWD, and not being confounded by chronic disease. Finally, HDGF has physical characteristics that increase its suitability as a clinical analyte. As shown in Figure E1, HDGF was stable over repeat freeze–thaw cycles and

thermostable for days at room temperature. There were also no significant differences in serum or plasma levels.

The primary limitation of this study is the small sample size of the cohorts, a common difficulty in studying any rare disease, in particular PAH. However, even with the relatively small sample size, a statistically significant difference was observed in serum HDGF levels. Also, the retrospective nature of the analysis is a limitation because, although the specimen and clinical data were collected prospectively, there could have been a lag between serum collection and clinical data collection, such as the NYHA-FC assessment, 6MWD, and hemodynamic data. Remaining questions, as described above, include HDGF as a diagnostic marker of PAH in at-risk groups, the role of HDGF levels in predicting therapeutic response, and the developmental regulation of HDGF.

In conclusion, HDGF represents a potential new PAH clinical indicator of disease severity and survival that may have possible distinct clinical advantages over NTproBNP. Whether it plays a role in the disordered pathologic cell growth of PAH or merely reflects this pathogenic state is unknown and will be a focus of future work. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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