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ORIGINAL RESEARCH

Angiostatic Peptide, Endostatin, Predicts Severity in Pediatric Congenital Heart Disease–Associated Pulmonary Hypertension

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BACKGROUND: Endostatin, an angiogenic inhibitor, is associated with worse pulmonary arterial hypertension (PAH) outcomes in adults and poor lung growth in children. This study sought to assess whether endostatin is associated with disease severity and outcomes in pediatric PAH.

METHODS AND RESULTS: Serum endostatin was measured in cross-sectional (N=160) and longitudinal cohorts (N=64) of pediatric subjects with PAH, healthy pediatric controls and pediatric controls with congenital heart disease (CHD) (N=54, N=15), and adults with CHD associated PAH (APAH-CHD, N=185). Outcomes, assessed by regression and Kaplan-Meier analysis, included hemodynamics, change in endostatin over time, and transplant-free survival. Endostatin secretion was evaluated in pulmonary artery endothelial and smooth muscle cells. Endostatin was higher in those with PAH compared with healthy controls and controls with CHD and was highest in those with APAH-CHD. In APAH-CHD, endostatin was associated with a shorter 6-minute walk distance and increased mean right atrial pressure. Over time, endostatin was associated with higher pulmonary artery pressure and pulmonary vascular resistance index, right ventricular dilation, and dysfunction. Endostatin decreased with improved hemodynamics over time. Endostatin was associated with worse transplant-free survival. Addition of endostatin to an NT-proBNP (N-terminal pro-B-type natriuretic peptide) based survival analysis improved risk stratification, reclassifying subjects with adverse outcomes. Endostatin was secreted primarily by pulmonary artery endothelial cells.

CONCLUSIONS: Endostatin is associated with disease severity, disease improvement, and worse survival in APAH-CHD. Endostatin with NT-proBNP improves risk stratification, better predicting adverse outcomes. The association of elevated endostatin with shunt lesions suggests that endostatin could be driven by both pulmonary artery flow and pressure. Endostatin could be studied as a noninvasive prognostic marker, particularly in APAH-CHD.

Key Words: angiogenesis ■ biomarkers ■ children ■ proteomics ■ pulmonary vascular disease

Pulmonary arterial hypertension (PAH) is a severe disease characterized by increased pulmonary vascular resistance (PVR) and pulmonary vasculature remodeling, leading to heart failure and death.[1] The 6th World Symposium on Pulmonary Hypertension defines Group 1 PAH as a mean pulmonary arterial pressure (mPAP) >20 mm Hg, a pulmonary vascular resistance index (PVRi) ≥3WU×m^2, and a pulmonary

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CLINICAL PERSPECTIVE

What Is New?

- Although cardiac biomarkers such as NT-proBNP (N-terminal pro-B-type natriuretic peptide) provide reliable information about outcomes in patients with pulmonary hypertension, endothelial biomarkers involved in the altered angiogenesis and right heart failure may improve detection of disease progression and outcome prediction.

What Are the Clinical Implications?

- This study demonstrates that endostatin can be used as a noninvasive biomarker of disease severity and survival in children with pulmonary arterial hypertension, especially when associated with congenital heart disease, and improves outcome prediction when combined with the current clinical biomarker NT-proBNP.

Nonstandard Abbreviations and Acronyms

6MWD	6-minute walk distance
APAH-CHD	pulmonary arterial hypertension associated with congenital heart disease
CHC	Children's Hospital of Colorado
mPAP	mean pulmonary arterial pressure
PAH	pulmonary arterial hypertension
PAHB	PAH Biobank

capillary wedge pressure <15 mm Hg.[1] Understanding pediatric PAH is challenging because of limited pediatric-specific data and developing pulmonary vasculature. Pediatric PAH is distinct from adult PAH, with a larger prevalence of idiopathic PAH, heritable PAH, genetic syndromes, and disease–associated PAH, particularly PAH associated with congenital heart disease (APAH-CHD).[1]

APAH-CHD is unique because PAH often develops due to a shunt between the systemic and pulmonary circulations.[2] Distinguishing pulmonary vascular disease in the setting of CHD from right ventricular hypertension due to shunts or valvular obstruction is difficult; although cardiac catheterization is diagnostic, it is invasive and diagnoses only manifest pulmonary vascular disease but cannot detect early changes. The longer a high-pressure/high-volume shunt remains untreated, the more damage the pulmonary vasculature accrues.[3] Thus, identifying noninvasive biomarkers of pulmonary vascular disease, particularly those that are associated with clinical changes and outcomes, is critical.

Endostatin, an angiogenic inhibitor, is a fragment of collagen-XVIIIa1.[4] Expression of collagen-XVIIIa1 localizes predominantly to vasculature, including the coronary arteries, and pulmonary vasculature[5]; Hoffman identified collagen-XVIIIa1 and endostatin in the media and intima of pulmonary vessels from PAH explanted lungs and associated endostatin levels with idiopathic PAH severity.[6] Genetic variants of endostatin have been linked to PAH disease severity and outcomes in adults.[7] Endostatin has also been studied in infants with lung disease–associated pulmonary hypertension (World Symposium on Pulmonary Hypertension Group 3) where elevated endostatin was associated with worse disease.[8–10]

The relationship between endostatin, disease severity, and mortality in pediatric PAH, particularly APAH-CHD, is not well studied. This study examined the associations between endostatin and hemodynamics, clinical variables, and outcomes in pediatric PAH using a cross-sectional cohort with longitudinal validation. Additionally, this study assessed endostatin as a marker of clinical improvement and worse outcomes in addition to the standard clinical laboratory marker, NT-proBNP (N-terminal pro--B-type natriuretic peptide).

METHODS

Analytic Cohorts

Two cohorts of pediatric (age <21) Group 1 subjects with PAH were used: a cross-sectional cohort from the Cincinnati Children's Hospital Medical Center Pulmonary Arterial Hypertension Biobank (PAHB) and a longitudinal cohort from the Children's Hospital of Colorado (CHC). Additionally, a cross-sectional cohort of adults with APAH-CHD from the Pulmonary Arterial Hypertension Biobank (Adult-PAHB) was used to further evaluate findings in APAH-CHD. The pediatric subjects with PAH were compared with 2 control cohorts: a group of healthy children and a group with CHD without PAH. All subjects with CHD (both pediatric and adult) had repaired, biventricular heart disease. The institutional review board approved each study with informed consent at the following locations: Johns Hopkins University, Cincinnati Children's Hospital, Children's Hospital Colorado, and all other PAHB participating centers. Because Trisomy 21 is associated with increased endostatin,[11,12] all subjects with Trisomy 21 were excluded from analysis. Data and analytic methods are available on request.

Pulmonary Arterial Hypertension Biobank

The PAHB, funded by the National Heart, Lung, and Blood Institute, serves as a resource for the PAH

research community, with 5 pediatric and 34 adult enrolling centers. Data include biological samples, genetic data, and clinical data (www.pahbiobank.org). Blood specimens were collected at enrollment and stored at the University of Cincinnati. Clinical parameters were obtained at the time of PAH diagnosis but were not necessarily concurrent with PAHB enrollment. Clinical data included age, sex, medical therapy, World Symposium on Pulmonary Hypertension Group 1 subgroups, World Health Organization functional class (functional class), 6-minute walk distance (6MWD), invasive hemodynamics, and death, transplant, Pott's shunt, or atrial septostomy. De-identified biological samples and clinical data were analyzed for the pediatric group (PAHB, N=160) and the adults with APAH-CHD (Adult-PAHB, N=185). Subjects were followed prospectively for up to 5 years with events captured including death, transplant, or palliative shunt (Table 1).

Children's Hospital of Colorado

Pediatric subjects with PAH from CHC were enrolled between November 2006 and September 2019 (N=64, 187 samples) with blood samples and clinical data, and echocardiographic measures collected concurrently. All subjects presented with Group-1 pulmonary hypertension. Data concerning clinical assessments and hemodynamics were previously published.[13] The CHC cohort was used solely for longitudinal analysis, because of crossover with the PAHB.

Healthy Controls

Healthy controls included children (N=54, age <21) presenting for elective surgery at Johns Hopkins Children's Center with discarded blood samples. Healthy controls had no chronic conditions and were diagnosed only with their presenting surgical complaint (Table S1). Controls with cardiac or pulmonary conditions or history thereof or any condition requiring daily medication were excluded. Samples were collected with Johns Hopkins Institutional Review Board approval and waiver of informed consent.

Congenital Heart Disease Controls

Subjects with CHD (n=15, age <21) without PAH were recruited from the Johns Hopkins Pediatric and Congenital Heart Center. Subjects presented for clinically indicated diagnostic or interventional (atrial/ventricular septal defect and patent ductus arteriosus device closures) cardiac catheterization between 2011 and 2015. Subjects were included if they had biventricular CHD and did not have pulmonary hypertension determined during catheterization. Blood samples were collected at vascular access, before placement of intracardiac catheters. Details of CHD types are provided in Table S2.

Laboratory Analysis

Serum samples were assayed from the PAHB (n=174), CHC (n=64, 187 samples), healthy controls (n=54), and controls with CHD (n=15). Primary cell lines were obtained from the Cardiovascular Medical Research and Education Fund Pulmonary Hypertension Breakout Initiative Penn Cell Center (Philadelphia, PA) from transplanted patients with severe PAH (n=25) and control cells from non-transplanted donor lungs (n=11). All samples were assayed on the same ELISA platform. Cell culture methods are detailed in Data S1 and have been previously published.[14]

Echocardiographic Variables

Echocardiography was performed and interpreted by the CHC echocardiography laboratory as part of clinical evaluation. Variables included right ventricular function (qualitative function (normal, mild, moderate, or severe), right ventricle fractional area change, right ventricle tissue Doppler imaging S', tricuspid annular plane systolic excursion, and tricuspid annular plane systolic excursion *Z* score, right ventricular size and dilation (right ventricular end diastolic area and end systolic area), right ventricular hypertension (tricuspid regurgitation velocity, septal flattening), pulmonary artery end diastolic pressure, pulmonary artery acceleration time, right ventricular ejection time, and pulmonary artery acceleration time/right ventricular ejection time). Detailed echocardiographic methods are described in Data S1.

Statistical Analysis

Continuous variables are presented as means with SDs or medians with interquartile range based on normality of the data. Comparisons were made between variables with *t* tests, Kruskal-Wallis H tests, or Mann-Whitney *U* tests as appropriate. Endostatin concentrations were log transformed for regression owing to nonnormality.

Associations of endostatin with clinical variables were analyzed using linear/logistic regression models adjusted for age and sex overall and by PAH subgroups. Pulmonary hemodynamics (PAP, PVR, PVRi) were additionally adjusted for cardiac output. Clinical variables included invasive hemodynamics (mean right atrial pressure, mPAP, pulmonary capillary wedge pressure, PVR, PVRi, cardiac output), echocardiographic measures, World Health Organization functional class, and 6MWD. Pulmonary hemodynamic measures (mean right atrial pressure, mPAP, PVR, PVRi) were adjusted for cardiac output to assess the relationship of endostatin and pulmonary vascular hemodynamics, independent of function. In the CHC, a mixed model longitudinal linear/logistic

Table 1. Demographics and Clinical Characteristics of PAH Cohorts at Enrollment

	PAHB*	CHC*	Adult biobank APAH-CHD (Adult PAHB)	Healthy controls	CHD controls
Demographics					
Subjects, n	160	64	185	54	16
Age, y	13 (8–17)†	5 (3–9)†	38 (20–55)	10 (6–14)	8 (5–17)
Sex, female, n (%)	127 (79)	25 (39)	138 (75)	22 (41)	8 (53)
Weight, kg	42.5 (19–55)†	16.5 (11 –28.8)†	58 (34–75)	…	34 (19–62)
Height, cm	149 (118–160)	104 (84–123)	157 (142–167)	…	…
Body surface area, m^2	1.2 (0.7–1.4)	0.7 (0.48–1)	1.47 (1.1–1.69)	…	…
World Health Organization functional class, n I/II/III/IV/ missing (%III/IV)	18/43/46/11/42 (35)	18/12/11/5/19(25)	14/54/52/8/57 (32)	…	…
6-minute walk distance, m (n) (%)	423 (79)	426 (22)	397 (127)	…	…
Deaths, n (%)	6 (3)	1 (1.6)	30 (11)	…	…
Events, n (%)	16 (10)	12 (18)	30 (11)	…	…
Etiology, n (%)	…	…	…	…	…
APAH-Overall	72 (41)	28 (44)	…		
APAH-CHD	61 (33)	28 (44)	185		
IPAH	81 (51)	26 (41)	…		
FPAH	11 (7)	6 (9)	…		
Other	7 (4)	4 (6)	…		
No. of visits	…	2 (1–3)	…	…	…
Time between visits, mo	…	16 (11–27)	…	…	…
Time from enrollment to censor (mo)	43 (28–56)	38 (18–72)	38 (24–52)	…	…
Biomarker values					
Endostatin, ng/mL	29 (22.3–36.5)	24.59 (19.1–35.2)	33 (24.7–43)	21 (18–26.9)	19.5 (13–25)
N-terminal pro-B-type natriuretic peptide, pg/mL	201 (75–408)	243 (135–599)	467 (231–1698)	99 (49–172)	65 (29–133)
Endostatin values by PAH subtype*					
IPAH	27 (23.3–34.9) (n=57)	26.1 (20.8–35.7) (n=26)	…	…	…
FPAH	30.5 (20.8–35.9) (n=7)	19.6 (18.8–27.2) (n=6)	…	…	…
APAH-CHD	32.7 (23.6–41.8) (n=52)	28.8 (22.8–42.5) (n=26)	33 (24–43) (n=185)	…	…
Ventricular shunt	42.7 (36.5–51.9) (n=16)	48.9 (23.7–60.6) (n=3)	36 (29.3–51.9) (n=42)	…	…
Atrial shunt	33.1 (22.8–45.3) (n=12)	30.7 (24.2– 41.5) (n=8)	31.7 (24.3–42.3) (n=94)	…	…
Complex CHD	29.3 (24.9–35.5) (n=24)	25.5 (21.5–33.7) (n=15)	27.3 (27.4–38.9) (n=49)	…	…
APAH-Other	28.2 (20.4–38.6) (n=13)	…	…		
Hemodynamics					
Mean right atrial pressure, mm Hg	7 (5–9)	6 (5–8)	7 (5–10)	…	6 (4–8)
Mean pulmonary arterial pressure, mm Hg†	55 (42–65)	37.5 (29–52.5)	54 (41–65)	…	16 (14–17)
Pulmonary capillary wedge pressure, mm Hg	9 (7–11)	8 (7–9)	10 (7–12)	…	8 (7–11)
PVR, Wood units	13.8 (9.2–21.5)	11 (6.8–17)	11 (7.2–17.8)	…	0.96 (0.5–1.3)

(*Continued*)

Table 1. (Continued)

	PAHB*	CHC*	Adult biobank APAH-CHD (Adult PAHB)	Healthy controls	CHD controls
PVR index, Wood units×m^2	17.5 (9.6–33)	7.9 (4.7–13.5)	8.9 (4.7–15.8)	…	1.1 (0.9–1.45)
Cardiac output, L/min	3.3 (2.1–4.1)	3.3 (2.4–4.5)	3.65 (2.5–4.9)	…	4.8 (3.7–5.7)
Cardiac index, L/min/m^2	3.6 (2.9–4.9)	3.6 (3.1–4.5)	2.9 (2.1–3.9)	…	4.23 (3.4–4.9)
Echocardiographic measures					
Tricuspid regurgitation peak gradient, mm Hg	…	65 (38.4–77)	…	…	…
RV end diastolic area, mL/m^2	…	19.1 (14.4–23.4)	…	…	…
RV end systolic area, mL/m^2	…	12 (8.8–16.9)	…	…	…
RV fractional area change, %	…	37.5 (29.9–39.8)	…	…	…
RV tissue Doppler imaging S', cm/s	…	12 (9–14)	…	…	…
TAPSE, cm	…	1.79 (1.4–2.2)	…	…	…
TAPSE *Z* score	…	–1.28 (–3.7 to 0.6)	…	…	…
Pulmonary artery end diastolic pressure, mm Hg	…	22 (6.8–38)	…	…	…
Pulmonary artery acceleration time, msec	…	70.8 (53.2–85)	…	…	…
RV ejection time, msec	…	275 (220–305)	…	…	…
Pulmonary artery acceleration time/right ventricular ejection time ratio	…	0.27 (0.23–0.31)	…	…	…
Therapies, n (%)					
Phosphodiesterase-5 inhibitor	147 (92)	38 (70)	152 (82)	…	…
Endothelin receptor antagonist	103 (64)	17 (31)	117 (63)	…	…
Intravenous/subcutaneous prostacyclin	60 (37.5)	16 (29)	37 (20)	…	…
Calcium channel blocker	28 (17.5)	3 (5.5)	22 (12)	…	…

All data presented as median (interquartile range) unless otherwise specified. Events include death, lung transplant, or Pott's shunt or atrial septostomy. APAH indicates associated PAH; APAH-CHD, congenital heart disease–associated PAH; CHC, Children's Hospital Colorado; FPAH, familial PAH; IPAH, Idiopathic PAH; PAH, pulmonary arterial hypertension; PAHB, PAH biobank; PVR, pulmonary vascular resistance; RV, right ventricle; and TAPSE, tricuspid annular plane systolic excursion.

*Subjects enrolled in both PAHB and CHC removed from PAHB for comparison.

†$P < 0.05$ between groups.

regression, adjusted for age and sex and accounting for multiple time points per subject (using a random intercept for each subject to account for repeated measures), was performed. Echocardiographic measures evaluating the right ventricle were additionally adjusted for PVRi to assess right ventricular function independent of afterload. To assess endostatin as a marker of clinical improvement, hemodynamics were compared between visits 1 and 2 in the CHC cohort. Subjects with a 10% or greater improvement in the majority of hemodynamic variables (10% decrease in mPAP, PVRi, and stable or improved cardiac output) were listed as improved. Endostatin levels in improved subjects were compared between visits 1 and 2 to see if endostatin changed with improved hemodynamics.

Relationships between endostatin and time to event (earliest event of either death, lung transplant, Pott's shunt, or atrial septostomy) were studied in the PAHB and CHC cohorts using Kaplan-Meier analysis with dichotomization based on the median endostatin. They were further explored using Cox proportional hazard models, with endostatin as a continuous variable and adjustment for age and sex.

Endostatin was evaluated in addition to the clinical standard, NT-proBNP, to determine if it added information about clinical worsening. Subjects were divided into risk groups based on their NT-proBNP levels and

then risk-assessed in each group based on endostatin dichotomized at the median. Methods comparing NT-proBNP and endostatin are in Data S1. Time to death, transplant, or palliative shunt was assessed using Kaplan-Meier analysis.

A 2-sided *P* value <0.05 was considered statistically significant. Statistical analyses were conducted with Stata (Version 15.1, StataCorp, College Station, TX) and R/R-studio (version 1.3.1093, RStudio Team (2020). Boston, MA, URL http://www.rstudio.com/.).

RESULTS

Patient Demographics

Demographic characteristics are shown in Table 1. PAHB subjects were predominantly female and older, whereas CHC subjects were predominantly male and younger. The age distribution of pediatric control subjects was not significantly different from the PAHB. Most pediatric PAHB subjects included idiopathic PAH (51%) or disease-associated PAH (41%), especially APAH-CHD (33%). Similarly, the CHC cohort predominantly included idiopathic PAH (41%) or APAH-CHD (44%). The types of CHD in each group is detailed in Table S2.

Both the PAHB and CHC subjects had moderate to severe PAH with a median mPAP of 55 mm Hg and 37.5 mm Hg respectively (Table 1). The adult PAHB was similar with a median mPAP of 54 mm Hg and PVRi of 8.9WU×m^2 (Table 1). The 2 pediatric cohorts had similar 6MWD and World Health Organization functional class.

Endostatin Levels in PAH, PAH Subtypes, and Controls

Endostatin was significantly different between subjects with PAH and healthy controls, with a median endostatin of 21 ng/mL in healthy controls and a median of 29 ng/mL in PAHB subjects (*P*<0.0001, Figure 1A). Subjects with idiopathic PAH had lower endostatin (Table 1, Figure 1A; 26.6 ng/mL) compared with subjects with APAH-CHD (Figure 1A; 32.8 ng/mL, *P*=0.0025). Those with a ventricular septal defect had the highest endostatin (Figure 1B; 42.7 ng/mL, *P*=0.001). In the CHC, the median endostatin was 24.6 ng/mL (Table 1). Endostatin was again higher in the APAH-CHD subgroup (Figure 1A; 32.4 ng/mL), with the highest concentrations in those with a ventricular septal defect (Figure 1C, 48.9 ng/mL), although this did not reach significance. In the Adult-PAHB, the median endostatin for all subjects with APAH-CHD was 33 ng/mL, and those with a ventricular septal defect had the highest endostatin (Figure 1D; 35.6 ng/mL, *P*=0.03).

Endostatin Associations With Hemodynamics and Functional Measures

The relationship between endostatin and hemodynamic changes is shown with adjusted regression coefficients (age, sex, cardiac output) for the overall cohorts in Table 2; the relationship was most significant in the APAH-CHD subgroups (Table 3). In adjusted regression in the APAH-CHD subgroup of the PAHB (Table 3), higher endostatin was associated with higher mean right atrial pressure and shorter 6MWD. In the APAH-CHD subgroup of the CHC cohort (Table 3), higher endostatin was associated with higher mPAP, PVR, and PVRi. In the Adult-PAHB cohort, higher endostatin was associated with higher mean right atrial pressure and shorter 6MWD (Table 3). Endostatin was not significantly associated with pulmonary capillary wedge pressure, cardiac output/index, or functional class in either cohort. There was a trend toward significant hemodynamic associations between endostatin and both complex CHD and atrial septal defect subjects, but with small sample size, this was not consistent (Tables S3 through S5).

Endostatin Associations With Echocardiographic Measures

In the longitudinal CHC cohort, endostatin was associated with echocardiographic measures of right ventricular dilation, higher pulmonary artery pressure, and right ventricular dysfunction overall and within the APAH-CHD subgroup (Table 4). In the overall cohort, the odds of ventricular dysfunction were 7 times higher for each log-unit increase in endostatin (95% CI, 1.75–29; *P*=0.006). Subjects with higher endostatin also had increased right ventricular end diastolic area, end systolic area, and pulmonary artery end diastolic pressure. In the APAH-CHD group, the odds of ventricular dysfunction were 48 times higher for each log unit increase in endostatin (95% CI, 1.1–2119; *P*=0.04, Table 4).

Endostatin as a Marker of Clinical Improvement

To assess whether endostatin concentrations changed with clinical changes over time, endostatin was assessed in CHC subjects with APAH-CHD who demonstrated clinical improvement (N=15, Figure 2). Overall, endostatin decreased in subjects who had hemodynamic improvement in mPAP and PVR in the absence of declining cardiac output; median endostatin decreased from 31 ng/mL to 22 ng/mL (*P*=0.001, Figure 2A).

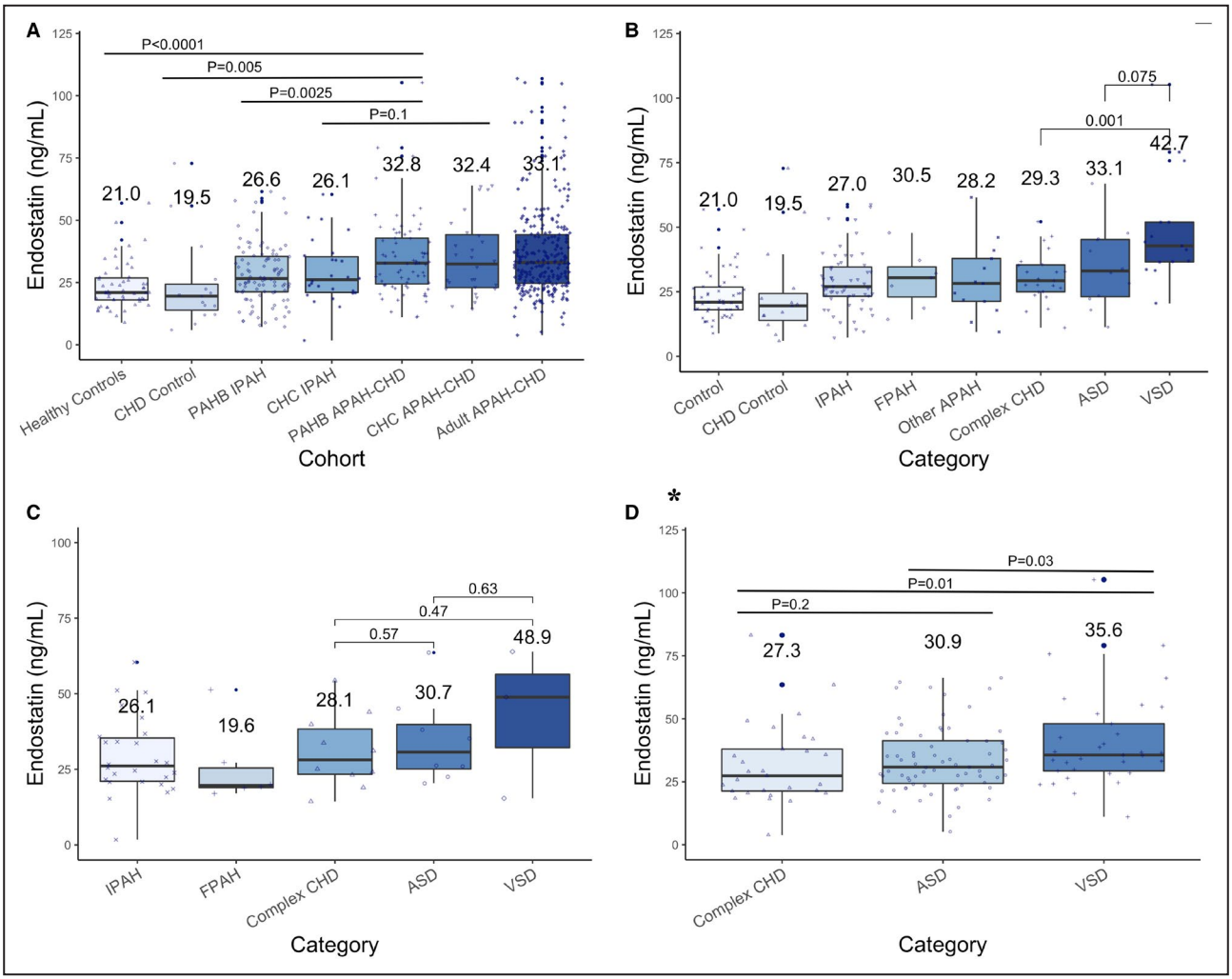

Figure 1. Endostatin concentration by cohort and PAH type.
Box and whisker plots (median and interquartile range) showing endostatin concentration by control groups and PAH (**A**) Endostatin concentration in control groups, PAHB IPAH, CHC IPAH, PAHB APAH-CHD, CHC APAH-CHD, and Adult APAH-CHD. **B**, Endostatin PH by control groups, subtype, and cardiac lesion in PAHB. **C**, Endostatin concentration by subtype and cardiac lesion in CHC. **D**, Endostatin concentration by cardiac lesion in Adult PAHB (only APAH-CHD subjects). *Subjects enrolled in both Pediatric PAHB and CHC removed from Pediatric PAHB. All APAH-CHD subjects have repaired CHD. Jitter plot demonstrates individual data points. APAH indicates associated PAH; APAH-CHD, congenital heart disease–associated PAH; ASD, atrial septal defect; CHC, Children's Hospital Colorado; FPAH, familial PAH; IPAH, idiopathic PAH; PAH, pulmonary arterial hypertension; PAHB, PAH biobank; and VSD, ventricular septal defect.

Endostatin and NT-proBNP Associations With Clinical Worsening

Kaplan-Meier analysis (Figure 3) demonstrated that high endostatin was associated with worse outcomes (death, lung transplant, palliative shunt) in both the PAHB and CHC (log rank *P*=0.003 and 0.001 respectively). In the PAHB, each log-unit higher endostatin was associated with an ≈5-fold increased risk of events, after adjustment for age and sex (hazard ratio [HR], 5.0; 95% CI, 1.7–15.0; *P*=0.004). The unadjusted HR in the CHC was 43 (95% CI, 1.6–1134; *P*=0.024) for a log increase in endostatin but was underpowered for adjusted analysis.

The association of endostatin, NT-proBNP, and clinical outcomes was explored to evaluate if endostatin could improve risk discrimination (Figure 4). A high risk (>median) NT-proBNP captured 13 of the 16 total events (81% of events). Similarly, a high risk (>median) endostatin captured 13 of the 16 total events (81% of events). Notably, each marker, endostatin and NT-proBNP, missed a different group of subjects when used individually. Combining both NT-proBNP and endostatin generated 3 categories: low risk (low- risk endostatin and NT-proBNP), moderate risk (high-risk endostatin with low-risk NT-proBNP OR low-risk endostatin with high-risk NT-proBNP) and high risk (high-risk

Table 2. Age-, Sex-, and Cardiac Output-Adjusted Linear/Logistic Regressions of Endostatin and Continuous Clinical Variables in the PAHB and CHC

	Pediatric PAHB (n=160)	CHC (n=64)
Mean right atrial pressure, mm Hg	2.2 (0.04–4.3, 0.04)*	0.7 (–0.24 to 1.6, 0.1)
Mean pulmonary arterial pressure, mm Hg	–0.02 (–6.1 – 6, 0.9)	4.2 (–0.4 to 8.8, 0.07)
Pulmonary capillary wedge pressure, mm Hg	0.01 (–1 to 1.1, 0.9)	0.4 (–0.5 to 1.3, 0.4)
PVR, Wood units	–0.6 (–3.7 to 2.5, 0.7)	1.7 (–1.2 to 4.5, 0.3)
PVR index, Wood units×m^2	3.2 (–4.6 to 11, 0.4)	1.3 (–0.6 to 3.2, 0.2)
Cardiac output, L/min	–0.01 (–0.5 to 0.5, 0.9)	–0.04 (–0.5 to 0.4, 0.8)
Cardiac index, L/min*m^2	–0.3 (–0.85 to 0.3, 0.4)	0.13 (–0.3 to 0.6, 0.6)
6-minute walk distance, m	–54 (–110 to –0.4, 0.05)*	–38 (–76 to 0.8, 0.05)*
WHO-FC	–0.43 (–1 to 0.26, 0.2)	–0.01 (–0.8 to 0.7, 0.9)

All data presented as regression coefficient (95% CI, *P* value). WHO-FC analyzed by ordinal logistic regression. Cardiac output and cardiac index adjusted for only age and sex. PAH Biobank n=160, CHC n=64. CHC indicates Children's Hospital Colorado; PAHB, pulmonary arterial hypertension biobank; PVR, pulmonary vascular resistance; and WHO FC, World Health Organization Functional Class.

* $P \le 0.05$.

endostatin and high-risk NT-proBNP). Addition of endostatin reclassified subjects with low NT-proBNP who had events into a moderate-risk group (Figure 4). Thus, in the group with low-risk NT-proBNP and low-risk endostatin, there were no events (0% events). The moderate risk category had 6 events (37.5% of total events). Those with both high NT-proBNP and high endostatin had highest risk with 10 events (62.5% of total events). The combination of markers allowed 100% of events to be captured in either a moderate or high-risk category. This was confirmed by Kaplan-Meier analysis, which demonstrated a significant difference between risk groups based on combined high endostatin and NT-proBNP (Figure 4, *P*=0.0008).

To further explore this relationship, NT-proBNP levels were divided into 3 groups based on the interquartile range: low NT-proBNP (<200 pg/mL, interquartile range <50%), medium NT-proBNP (200–400 pg/mL, interquartile range 50%–75%), and high NT-proBNP (>400 pg/mL, interquartile range >75%). Events were studied by median endostatin using Kaplan-Meier analysis (Figure S1). Subjects with above median endostatin trended toward increased risk compared with those with below median endostatin in both the medium and high NT-proBNP groups. The increased risk was significant in the low NT-proBNP group (Figure S1, *P*=0.04).

Endostatin Secretion by Pulmonary Vascular Cells

Endostatin secretion was measured in pulmonary artery endothelial cells and smooth muscle cells from 25 subjects with severe PAH and 11 controls. Endostatin concentration in the conditioned media was

Table 3. Age-, Sex-, and Cardiac Output-Adjusted Linear/Logistic Regressions of Endostatin and Continuous Clinical Variables in the PAHB and CHC APAH-CHD Subgroups

	Pediatric PAHB: APAH-CHD (n=52)	CHC: APAH-CHD (n=22)	Adult PAHB: APAH-CHD (n=185)
Mean right atrial pressure, mm Hg	3.5 (1.6–5.4, 0.01)*	1.7 (–0.09 to 3.5, 0.06)	2.1 (0.9 to 3.3, 0.01)*
Mean pulmonary arterial pressure, mm Hg	–0.4 (–10 to 9.5, 0.9)	9.5 (5.2 to 14, <0.001)*	–3.6 (–8.6 to 1.5, 0.2)
Pulmonary capillary wedge pressure, mm Hg	1.1 (–0.6 to 2.8, 0.2)	0.9 (–0.6 to 2.5, 0.2)	0.9 (–0.4 to 2, 0.2)
PVR, Wood units	1.6 (–3 to 6.2, 0.5)	4.8 (1.8 to 7.8, 0.02)*	–0.4 (–2.6 to 1.8, 0.7)
PVR index, Wood units×m^2	0.3 (–12 to 13, 0.9)	2.3 (0.6 to 4, 0.009)*	4.8 (–0.9 to 10.6, 0.1)
Cardiac output, L/min	0.3 (–0.5 to 1.7, 0.4)	0.5 (–0.3 to 1.4, 0.2)	–0.02 (–0.6 to 0.6, 0.9)
Cardiac index, L/min*m^2	–0.4 (–1.5 to 0.7, 0.5)	0.4 (–0.8 to 1.5, 0.6)	0.2 (–0.5 to 0.9, 0.6)
6-minute walk distance, m	–122 (–219 to –24, 0.03)*	59 (0.7–117, 0.047)*	–47 (–88 to –6, 0.02)*
WHO-FC	–0.37 (–1.5 to 0.8, 0.5)	–0.2 (–0.9 to 0.5, 0.6)	0.39 (–0.13 to 0.92, 0.14)

All data presented as regression coefficient (95% CI, *P* value). WHO-FC analyzed by ordinal logistic regression. Cardiac output and cardiac index adjusted for only age and sex. PAHB with repaired APAH_CHD n=52, CHC n=22, Adult PAHB with repaired APAH-CHD n=185. APAH-CHD indicates congenital heart disease–associated PAH; CHC, Children's Hospital Colorado; PAH, pulmonary arterial hypertension; PAHB, pulmonary arterial hypertension biobank; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; and WHO FC, World Health Organization Functional Class.

* $P \le 0.05$.

Table 4. Age- and Sex-Adjusted Logistic/Linear Regressions of Endostatin and Echo Variables in the CHC Overall and APAH-CHD Subgroups (Pediatric APAH-CHD, N=22)

Right ventricular measures (adjusted for age, sex, PVRi) Logistic regressions	Odds ratio, overall	Odds ratio APAH-CHD
RV dysfunction	7.2 (1.75–29.3, 0.006)*	47.6 (1.1–2119, 0.04)*
Flattened septum	4.1 (0.6–28, 0.15)	0.8 (–0.0002 to 3018, 0.9)
Right ventricular measures (adjusted for age, sex, PVRi) Linear regressions	**Coefficient, overall**	**Coefficient, APAH-CHD**
Tricuspid regurgitation eak gradient (RV systolic pressure, mm Hg)	4 (–8.8 to 16.8, 0.5)	6.4 (–9.8 to 22.5, 0.4)
RV end diastolic area, mL/m^2	2.78 (0.11–5.5, 0.04)*	0.34 (–5.5 to 6.2, 0.9)
RV end systolic area, mL/m^2	3.3 (0.6–5.9, 0.018)*	–1.14 (–5.5 to 1.7, 0.3)
RV fractional area change, %	–3.1 (–6.6 to 0.45, 0.08)	–2.2 (–5.2 to 0.9, 0.16)
RV tissue Doppler imaging S', cm/s	–1.3 (–2.7 to 0.13, 0.07)	–0.85 (–3.2 to 1.5, 0.5)
Tricuspid annular plane systolic excursion, cm	–0.13 (–0.59 to 0.3, 0.5)	0.25 (–0.16 to 0.6, 0.2)
Tricuspid annular plane systolic excursion *Z* score	–1.5 (–3.5 to 0.53, 0.15)	1.46 (–0.9 to 3.8, 0.2)
Pulmonary artery measures (adjusted for age, sex) Linear regressions	**Coefficient, overall**	**Coefficient, APAH-CHD**
Pulmonary artery end diastolic pressure, mm Hg	14.1 (3.2–25.1, 0.01)*	19.5 (7.5–31.4, 0.001)*
Pulmonary artery acceleration time, msec	–6.7 (–16.7 to 3.22, 0.18)	–6.4 (–29 to 17, 0.6)
RV ejection time, msec	–18.9 (–42.8 to 5.1, 0.12)	–18.7 (–84 to 46, 0.6)
Pulmonary artery acceleration time/right ventricular ejection time ratio	–0.006 (–0.03 to 0.02, 0.6)	–0.02 (–0.07 to 0.03, 0.5)

All data presented as regression coefficient or odds ratio (95% CI, *P* value). APAH_CHD n=22. RV Dysfunction and septal flattening scored as none, mild, moderate, severe. Echo completed within 30 days of cath and serum sample. APAH-CHD indicates congenital heart disease–associated pulmonary arterial hypertension; CHC, Children's Hospital Colorado; PVRi, pulmonary vascular resistance index; and RV, right ventricle.

* $P \leq 0.05$

significantly greater in endothelial cells compared with smooth muscle cells in all cell lines (Table 5; Figure 5, $P<0.0001$). Endostatin secretion was higher in PAH cell lines compared with that in controls, although this did not reach statistical significance. Notably, endostatin secretion by PAH pulmonary artery endothelial cells into the conditioned media (35.3 ng/mL) was similar to serum endostatin secretion in subjects with PAH.

DISCUSSION

Endostatin, a potent angiogenic inhibitor, has been previously implicated in PAH.[7,15] To our knowledge, this is the most comprehensive pediatric PAH study to link endostatin with disease severity, clinical improvement, and outcomes and confirms that endostatin is produced by the pulmonary vascular endothelium.

This study demonstrated that endostatin levels were increased in subjects with PAH compared with healthy controls and highest in subjects with APAH-CHD compared with other PAH subtypes and controls without PAH with CHD. Endostatin was associated with worse hemodynamic and functional changes, especially in those with APAH-CHD, cross-sectionally and over time. These data are reinforced by echocardiographic measures that show higher endostatin was associated with increased pulmonary artery pressures and right ventricular dysfunction, after adjustment for afterload. Endostatin levels also decreased with improved hemodynamics over time, suggesting that it may be a good alternative marker of invasive hemodynamics. Cell conditioned media confirmed that endostatin is secreted by pulmonary artery endothelium in concentrations similar to the serum of subjects with PAH. Endostatin was further associated with time to death, lung transplant, or palliative shunt. Most important, addition of endostatin improved risk assessment based on NT-proBNP levels. Endostatin may be a valuable tool in the assessment of disease severity and clinical course as well as for the prediction of survival in pediatric subjects with PAH.

Endostatin has its strongest hemodynamic association in subjects with APAH-CHD. It is intriguing to consider whether the increase in endostatin results from damage to the pulmonary vasculature due to intracardiac shunts, injury from surgical repair, and other comorbidities. Other studies suggest that endostatin is a vasodilator and may compensate for increased pulmonary artery pressures.[16] Endostatin has been shown to inhibit VEGF (vascular endothelial growth factor), preventing VEGF mediated cell proliferation and migration, which are essential for lung development.[15] Development of pulmonary vasculature occurs in conjunction with alveolarization, a rapid process during the

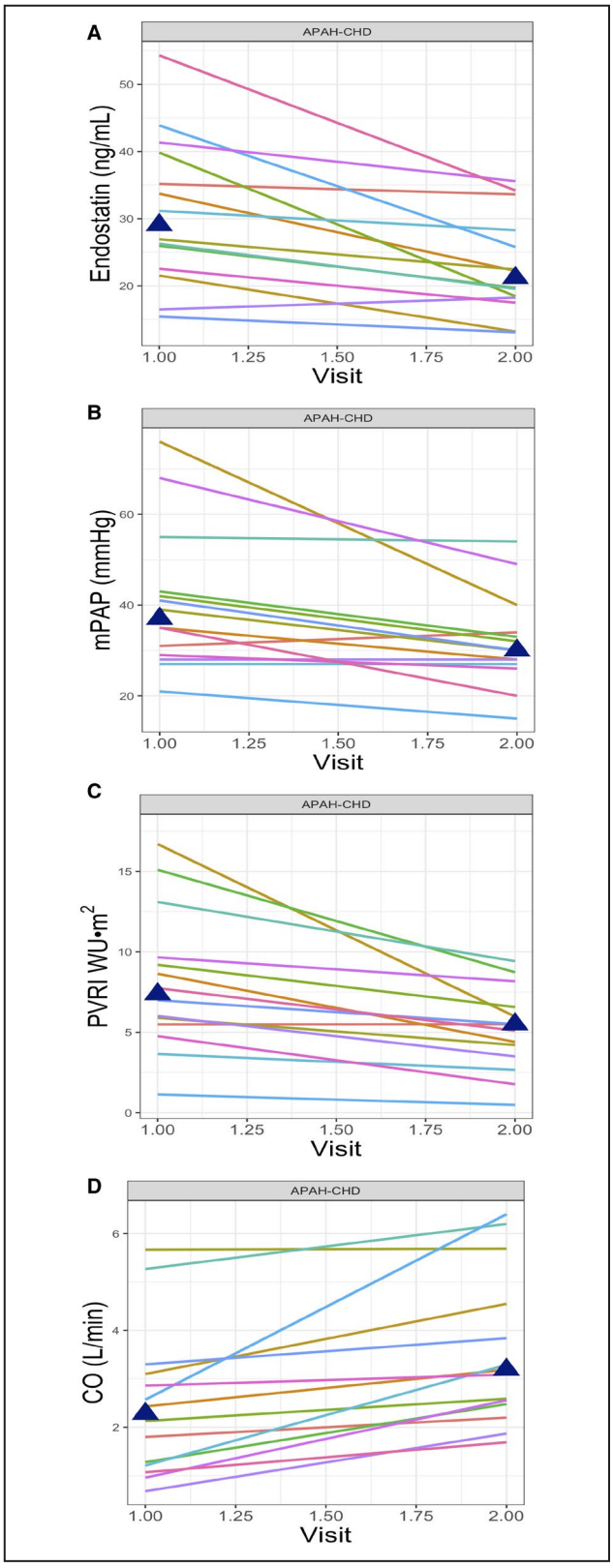

Figure 2. Change in endostatin and hemodynamics across visits.

A, Endostatin concentration at visit 1 and 2 in CHC APAH-CHD subjects who demonstrated hemodynamic improvement. Improvement determined by (**B**) decreased mPAP, (**C**) decreased PVRi, and (**D**) increased/stable CO. Color of lines represents individual subjects. APAH-CHD indicates congenital heart disease–associated PAH; CHC, Children's Hospital Colorado; CO, cardiac output; mPAP, mean pulmonary arterial pressure; PAH, pulmonary arterial hypertension; and PVRi, pulmonary vascular resistance index.

first 2 years of life that continues until adolescence, and is inhibited by injury including infection, prematurity, or high pressure.[17,18] Although endostatin is involved in fetal lung development, high concentrations postnatally, perhaps owing to elevated pulmonary artery pressures, may contribute to lung vascular injury.[18] Inhibiting vascular growth during alveolarization could mean that the lungs of children with PAH develop abnormally. Thus, even as interventions are developed to treat children with PAH and increase survival, children may suffer irreparable lung damage, leading to worse disease.

Right ventricular adaptation to increased workload from pulmonary hypertension is the primary determinant of outcomes, with right ventricle-pulmonary vascular uncoupling leading to decreased contractility, right ventricular dilation, and eventual failure.[19,20] Right ventricular adaption to PAH is especially salient in patients with CHD, who are at risk of ventricular dysfunction and dilation from chronically increased volume loads, valvular obstruction, surgical scar/patch material, altered ventricular interdependence, and abnormal flow dynamics due to anatomic defects. Prior studies have shown that capillary rarefaction, subsequent ischemia, and oxidative stress are hallmarks of decompensated right ventricular failure,[21] in animal models of right heart failure, loss of angiogenic markers such as hypoxia inducible factor-1α and VEGF as well as significantly decreased capillary density were features of the transition from compensated to decompensated right heart failure.[22] Bogaard further showed that high right ventricular pressure, as may be seen in CHD from either a shunt or obstruction, is insufficient to cause right heart failure and postulated that the abnormal angiogenesis characteristic of PAH has an effect on myocardial adaptation to increased workload.[23] Endostatin, as an angiogenic inhibitor, has been shown to decrease myocardial vascular growth resulting in scarce capillary density and decreased myocardial perfusion.[7,24–26] Thus, endostatin, secreted by the pulmonary artery endothelium, may cause injury to the pulmonary vasculature as well as a maladaptive response in the right ventricle, hastening the progression from right ventricular hypertrophy and dilation to right ventricular failure because of inadequate myocardial blood supply.

This study showed that adding endostatin to risk stratification based on NT-proBNP resulted in a more precise and accurate model. Endostatin and NT-proBNP in combination generated a moderate risk category and captured subjects whose adverse

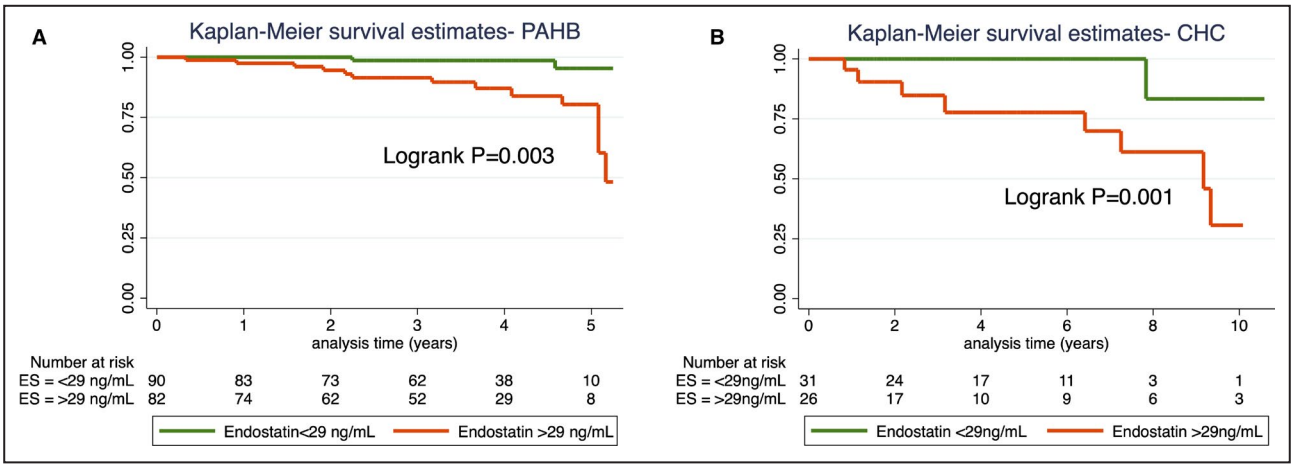

Figure 3. Survival curve by median endostatin.
Kaplan-Meier curve demonstrating survival (death, transplant, Pott's shunt, or atrial septostomy) by median endostatin (29 ng/mL) in (**A**) PAHB and (**B**) CHC cohorts. CHC indicates Children's Hospital Colorado; ES, endostatin; and PAHB, pulmonary arterial hypertension biobank.

outcomes would have been missed by either marker alone. NT-proBNP has been extensively described as an outcome predictor in pediatric PAH; Said and Ploegstra et al described an NT-proBNP <1200 pg/mL as a predictor of transplant free survival.[27,28] Kaplan-Meier analyses demonstrate that using both endostatin and NT-proBNP for risk stratification better predicts time to death, transplant, or palliative shunt (Figures 3 and 4). When stratifying NT-proBNP further, the improved risk prediction was greatest in the lowest risk NT-proBNP group, suggesting addition of endostatin could highlight patients whose risk would be missed by NT-proBNP alone.

Considering endostatin as a prognostic marker, which may reflect pulmonary vascular dysfunction with a potential effect on the right ventricle, is intriguing. Said et al described NT-proBNP as a surrogate prognostic and treatment marker in pediatric PAH, arguing that a surrogate treatment or prognostic marker should have biologic relevance, an association with treatment and survival, and that treatment induced changes are related to changes in survival.[27] Although our study design did not allow assessment of endostatin in response to treatment, we have shown that endostatin decreases significantly with improved hemodynamics. Thus, endostatin levels appear to decrease as subject health improves, suggesting its role as a surrogate marker for clinical improvement in lieu of more invasive methods. A prospective study would be needed to assess endostatin in relation to treatment and as a surrogate treatment goal.

Limitations

Limitations of this study include small size, although large in pediatrics, and missing data for some of the group (6MWD, echocardiogram measurements), thus underpowering some outcomes. Survival analysis was limited because there were only 16 events in the PAHB cohort and 12 events in the CHC cohort, making adjusted survival models more difficult to develop. There may be a delay between blood samples and clinical/hemodynamic data in the PAHB cohort. The concurrent serum samples and hemodynamics in the CHC cohort, where there is a stronger association between endostatin concentrations and pulmonary hemodynamic measures, supports the interpretation of the PAHB data despite the delay. A prior study by Damico et al demonstrated that an endostatin variant single-nucleotide polymorphism (chr21:45511195) was a genetically determined predictor of adverse outcomes in PAH; genetic data were available, but the single-nucleotide polymorphism in question was present in only 1 subject and thus were unable to be evaluated.[7] The study also analyzed both incident and prevalent cases; thus we cannot determine if endostatin is a cause or result of disease. Finally, although the cell culture results confirm the findings of prior studies, these cells were adult derived and were not from subjects with APAH-CHD. Further in vitro studies should look at endostatin secretion in the setting of high pressure or shear stress, as well as endostatin secretion in the myocardium and coronary circulation.

CONCLUSIONS

Studying APAH-CHD as a subgroup is important given the heterogeneity of CHD and the difficulty defining pulmonary hypertension in this population, particularly in more complex congenital heart defects. The majority of APAH-CHD subjects in this group had simpler lesions, ventricular/atrial septal defects, and all subjects had repaired biventricular hearts. Nonetheless,

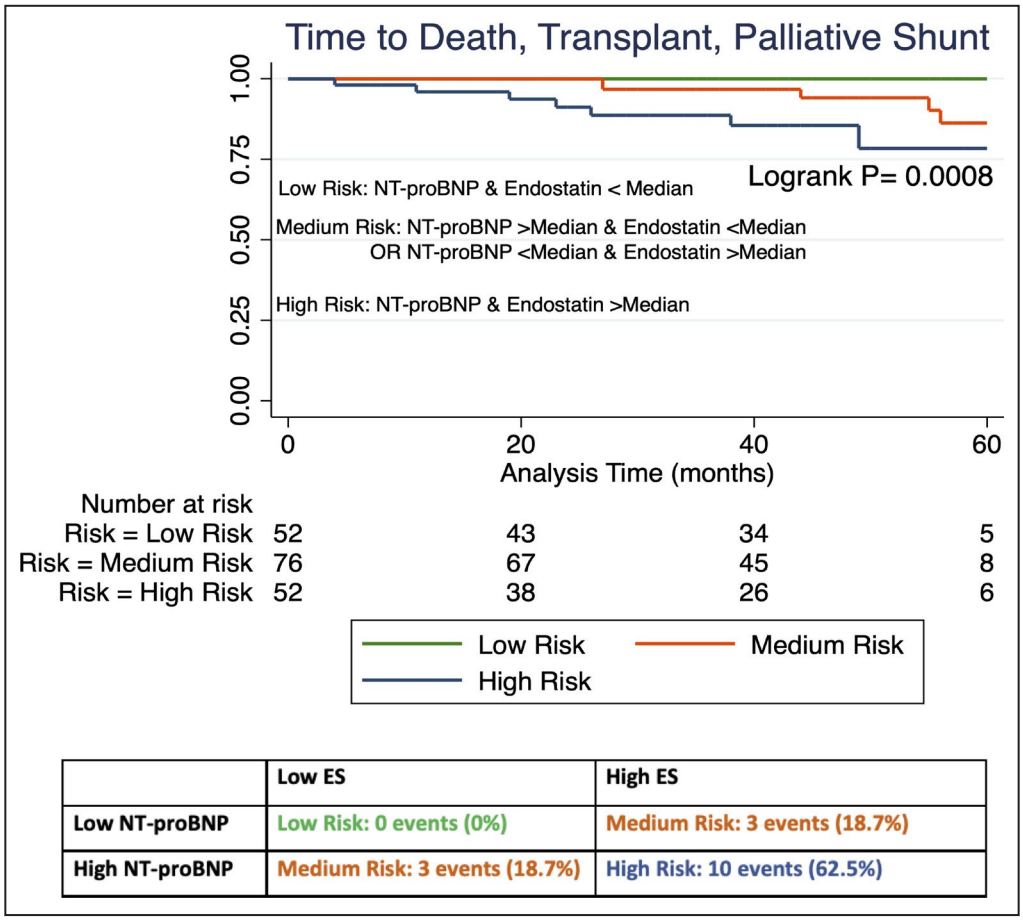

Figure 4. Survival curve by NT-proBNP and endostatin.
Kaplan-Meier curve demonstrating survival (death, transplant, Pott's shunt, or atrial septostomy) by low, medium, and high-risk NT-proBNP and endostatin dichotomized by median values (NT-proBNP 201 pg/mL; endostatin 29 ng/mL). Two-by-two table showing total events (percentage of total events) in each risk group. Difference in survival between groups (P=0.0008). ES indicates endostatin; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

the antiangiogenic effect of endostatin on both the heart and lung microvasculature could be applicable to more complex cardiac lesions and could be informative in cases where hemodynamics are difficult to interpret (single ventricle, surgical shunts/palliation). In this subgroup, following endostatin may give information on pulmonary vascular disease and maladaptive response to unrepaired heart disease or residual lesions, potentially guiding timing for intervention.

Table 5. Endostatin Secretion by Cell Type in PAH and Failed Donor Cells

	PAH cells	Failed donor cells
Pulmonary arterial endothelial cells, ng/mL	35.3 (16.5–45.8)	27.9 (26.4–35.0)
Pulmonary artery smooth muscle cells, ng/mL	7.7 (6.3–15.1)	5.9 (4.6–11.2)

All data presented as median (interquartile range). PAH indicates pulmonary arterial hypertension.

Endostatin was associated with worse functional measures, pulmonary vascular hemodynamics, echocardiographic measures, and survival in pediatric APAH-CHD. In addition, endostatin adds to NT-proBNP, the current clinical standard, for predicting adverse events. These observations suggest that serum endostatin could act as a pulmonary vascular biomarker to identify those who are at increased risk of developing PAH and predict poor outcomes, especially in APAH-CHD. Next steps include studying endostatin in patients with CHD to see if it could be used to guide therapy or intervention and evaluating the effect of endostatin on the right ventricular myocardium.

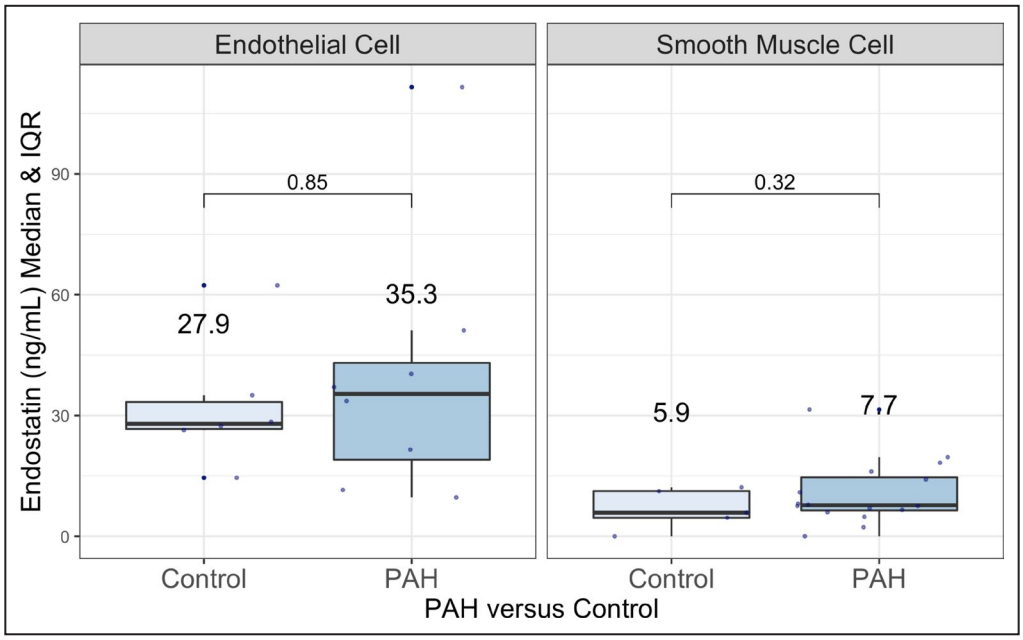

Figure 5. Endostatin in cell conditioned media.
Concentration of endostatin (ng/mL) in pulmonary artery endothelial cell and smooth muscle cell lines in severe pulmonary arterial hypertension. Cell types differ in endostatin concentration ($P < 0.0001$). IQR indicates interquartile range; and PAH, pulmonary arterial hypertension.

ARTICLE INFORMATION

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Disclosures

All authors confirm that they have no relevant financial disclosures.

Supplementary Material

Data S1
Tables S1–S5
Figure S1

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Supplemental Material

Data S1.

Supplemental Methods

Laboratory Analysis:

A custom electrochemiluminescent immunosorbent assay was developed (MesoScale Discovery, Gaithersburg, MD) by robotic printing capture antibodies (R&D Systems DIY1098, part 841455) and paired with an endostatin detection antibody and assay standards (R&D Systems DIY1098, part 841456) to quantify serum endostatin. Intraassay CV for endostatin was 2.38. NT-proBNP was previously measured in these cohorts using the same multiplex ELISA on capture antibody-spotted plates and was used for comparison to endostatin (28). Intraassay CV for NT-proBNP was 3.0%.

Cell Culture:

Primary pulmonary artery smooth muscle and endothelial cell lines were obtained from the Cardiovascular Medical Research and Education Fund PHBI Penn Cell Center (Philadelphia, PA). Cells were collected from transplanted patients with severe PAH (n=25), and control cells were collected from non-transplanted donor lungs (n=11). Methods for cell culture have been previously published (28). Briefly, cells were maintained at low passage number (passage 3-8) in a humidified 5% CO_2-supplemented incubator at 37°C (Napco 8000 DH, Thermo Scientific). Culture media was complete endothelial or smooth muscle cell medium from Lifeline Cell Technology (Vasculife VEGF-Mv Complete Medium (LL-0005) or Vasculife SMC Complete Medium (LL-0014), respectively). Cell conditioned media was obtained at 80% confluence, stored at -80°C, and subsequently assayed on the MSD multiplex ELISA platform using the above methods.

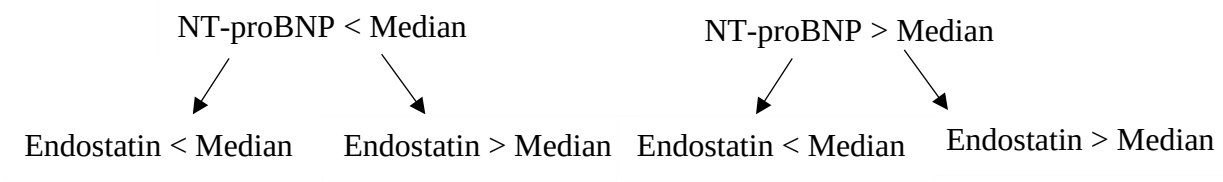
Echocardiographic variables:

Echocardiographic methods have been previously published (29). Measurement of Pulmonary Artery Acceleration Time (PAAT) and right ventricular ejection time (RVET) were performed subsequent to prior publication and were performed according to the echocardiography lab standard clinical protocol. Echocardiography was performed and interpreted by the CHC echocardiography lab which is an Intersocietal Accreditation Commission accredited lab. Echo images were only used if they were obtained within 30 days of blood sample. Images were obtained using an iE33 (Philips Ultrasound, Bothell, WA) or a Vivid 7 (GE Medical Systems, Milwaukee, WI) ultrasound machine. Digital images were obtained with appropriately sized transducers for each subject and analyzed using the Agfa Heartlab system (Mortsel, Belgium). Echocardiographic variables included measures of right ventricular function (qualitative right ventricular function (normal, mild, moderate, or severe), right ventricular fractional area change, right ventricular Tissue Doppler Imaging (TDI) S', tricuspid annular plane systolic excursion (TAPSE), and TAPSE Z score), right ventricle size and dilation (right ventricular end diastolic area, right ventricular end systolic area), right ventricular hypertension (tricuspid regurgitation, TR velocity, septal flattening), pulmonary artery end diastolic pressure, pulmonary artery acceleration time, right ventricular ejection time, and (PAAT/RVET)). Qualitative measures of right ventricular hypertension including septal flattening, and qualitative assessment of right ventricular function were assessed as either none, mild, moderate, or severe by the interpreting cardiologist and were studied as ordinal variables.

Comparison to NT-proBNP:

Endostatin was evaluated against the clinical standard, NT-proBNP, to determine if it added information about clinical worsening. NT-proBNP levels were evaluated in 2 ways. NT-

proBNP was dichotomized at the median (211 pg/mL), and time to event was evaluated based only on NT-proBNP by Kaplan Meier analysis showing significant increase in risk of event for subjects with NT-proBNP above the median. The two NT-proBNP groups were then compared based on endostatin dichotomized at the median (29.5 ng/mL). Kaplan-Meier analysis was conducted by comparing subjects with low NT-proBNP and low endostatin, low NT-proBNP and high endostatin, high NT-proBNP and low endostatin, or high NT-proBNP and high endostatin.



NT-proBNP was then divided into 3 groups based on interquartile range, Low NT-proBNP<200 pg/mL (IQR<50%), Medium NT-proBNP 200-400 pg/mL (IQR 50-75%), and High NT-proBNP>400 pg/mL (IQR >75%). Events in each NT-proBNP group were compared based on endostatin dichotomized at the median endostatin (29.5 ng/mL). Further Kaplan-Meier analyses were conducted within each of the three groups of NT-proBNP, with subjects dichotomized by the median value of endostatin.

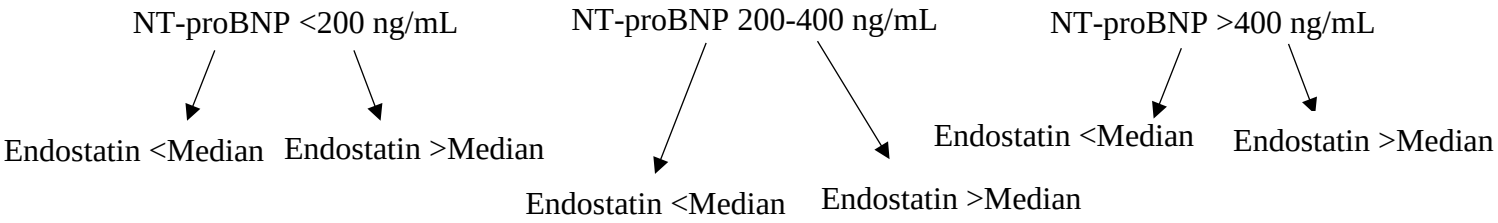


Table S1. Presenting Diagnosis of Healthy Controls.

	Healthy Controls
Orthopedic procedure (ACL repair, knee or ankle arthroscopy)	3
Abdominal Pain (endoscopy)	5
Dermoid or skin Cyst	5
Trauma/Wound repair	4
Urologic procedure (hypospadias, undescended testes, circumcision revision)	7
Hearing evaluation	2
Fracture repair	2
Unspecified Lesion	26

All data presented as number of subjects with presenting diagnosis.

Table S2. Congenital Heart Disease Lesions.

	PAHB	CHC	Adult PAHB	CHD Controls
ASD	18	8	94	9
VSD	15	4	42	5
PDA	8	2	12	
Tetralogy of Fallot	4	-	13	3
Atrioventricular Septal Defect	7	3	9	-
Truncus Arteriosus	1	-	3	-
Anomalous Pulmonary Venous Return	3	4	10	-
D-Transposition of the Great Arteries	3	1	5	-
Other complex Biventricular lesion	4	3	17	2

All data presented as number of subjects with lesion.

Some subjects listed in multiple categories if had multiple lesions, i.e. membranous VSD and secundum ASD or Atrioventricular Septal Defect and Truncus Arteriosus

**Complex biventricular lesions included double outlet right ventricle (DORV), lesions with pulmonary atresia, or lesions that included left sided heart obstruction such as coarctation of the aorta*

Table S3. Age- sex- and cardiac output- adjusted linear/logistic regressions of endostatin and continuous clinical variables in the Peds PAHB by CHD subtype.

	All PAH (n=160)	APAH-CHD (n=52)	ASD (n=11)	VSD (n=16)	Complex CHD (N=23)
Age, years	-0.8 (-2.5-0.9, *0.4*)	-1.5 (-4.3-1.4, *0.3*)	-5.6 (14.5-3.2, *0.2*)	1.4 (-3.6-6.3, *0.6*)	-2.3 (-7.4-2.7, *0.3*)
BSA, m^2	-0.01 (-0.2-0.1, *0.9*)	0.02 (-0.1-0.2, *0.7*)	-0.2 (-0.5—0.1, *0.3*)	-0.02 (-0.3-0.3, *0.9*)	0.1 (-0.09-0.4, *0.2*)
mRAP, mmHg	2.2 (0.04- 4.3, *0.04*)*	3.5 (1.6-5.4, *0.01*)*	2.4 (-3.2- 8, *0.4*)	3.9 (0.1- 7.7, *0.07*)	4.3 (0.9- 7.6, *0.02*)*
mPAP, mmHg	-0.02 (-6.1 – 6, *0.9*)	-0.4 (-10-9.5, *0.9*)	19.7 (-14- 53, *0.2*)	-3 (-17-10.8, *0.7*)	3.3 (-15- 22, *0.7*)
PCWP, mmHg	0.01 (-1- 1.1, *0.9*)	1.1 (-0.6-2.8, *0.2*)	4.6 (-0.8- 1, *0.15*)	-0.5 (-3.9- 2.9, *0.8*)	1.9 (-1.7- 5.5, *0.3*)
PVR, Wood units	-0.6 (-3.7- 2.5, *0.7*)	1.6 (-3-6.2, *0.5*)	9.8 (-3.6- 23, *0.2*)	1.3 (-6.6- 9, *0.8*)	0.03 (-7.2- 7.2, *0.9*)
PVRi, Wood units*m^2	3.2 (-4.6 -11, *0.4*)	0.3 (-12- 13, *0.9*)	-34 (-93 -24, *0.3*)	0.5 (-23- 24, *0.9*)	3 (-15- 22, *0.8*)
Cardiac output, L/min	-0.01 (-0.5- 0.5, *0.9*)	0.3 (-0.5-1.7, *0.4*)	0.7 (-2.3- 3.8, *0.7*)	-0.5 (-2- 1.2, *0.6*)	1.2 (-0.4- 2.7, *0.2*)
Cardiac index, L/min*m^2	-0.3 (-0.85-0.3, *0.4*)	-0.4 (-1.5-0.7, *0.5*)	1.4 (-3.5- 6.3, *0.6*)	-0.6 (-2- 0.9, *0.5*)	-0.09 (-2.4- 2.2. *0.9*)
6MWD, m	-54 (-110- -0.4, *0.05*)*	-122 (-219- -24, *0.03*)*	Inadequate n	Inadequate n	-125 (-208- -42, *0.03*)*
WHO-FC	-0.43 (-1-0.26, *0.2*)	-0.37 (-1.5-0.8, *0.5*)	0.9 (-4-6, *0.7*)	-5.6 (-13-2.5, *0.2*)	0.5 (-1.9-2.99, *0.7*)

All data presented as regression coefficient (95% CI, p value). WHO-FC analyzed by ordinal logistic regression.
**p<0.05*
Cardiac output and cardiac index adjusted for only age and sex.
Definition of abbreviations: PAH: pulmonary arterial hypertension; PAHB: PAH biobank; CHC: Children's Hospital Colorado; APAH: associated PAH; APAH-CHD: Congenital heart disease associated PAH; IPAH: Idiopathic PAH; FPAH: familial PAH; ASD: atrial septal defect; VSD: ventricular septal defect

Table S4. Age- sex- and cardiac output- adjusted linear/logistic regressions of endostatin and continuous clinical variables in the CHC Cohort by CHD subtype.

	All PAH (n=54)	APAH-CHD All (n=22)	ASD (n=8)	VSD (n=4)	Complex CHD (N=10)
Age, years	-0.39 (-1.6-0.9, *0.5*)	-0.7 (-2.9-1.5, *0.5*)	0.6 (-2-3.4, *0.7*)	-3.3 (-7.5-0.8, *0.1*)	-0.4 (-4-3.3, *0.8*)
BSA, m^2	-0.001 (-0.04-0.04, *0.9*)	-0.04 (-0.1-0.05, *0.4*)	-0.008 (-0.06-0.05, *0.7*)	0.2 (-0.03-0.4, *0.09*)	-0.2 (-0.3- -0.06, *0.005*)*
mRAP, mmHg	0.7 (-0.24-1.6, *0.1*)	1.7 (-0.09- 3.5, *0.06*)	0.8 (-1.6- 3.2, 0.5)	0.6 (-1.4- 2.6, *0.6*)	3.5 (0.3- 6.7, *0.03*)*
mPAP, mmHg	4.2 (-0.4- 8.8, *0.07*)	9.5 (5.2- 14, *<0.001*)*	11 (5.6- 15.8, *<0.001*)*	-10.4 (-24- 2.8, *0.13*)	7.7 (1.2- 14, *0.02*)*
PCWP, mmHg	0.4 (-0.5- 1.3, *0.4*)	0.9 (-0.6- 2.5, *0.2*)	-0.7 (-3.3- 2, *0.6*)	0.5 (-2- 2.9, *0.7*)	2.9 (0.5- 5.3, *0.017*)*
PVR, Wood units	1.7 (-1.2- 4.5, *0.3*)	4.8 (1.8- 7.8, *0.02*)*	3.7 (-1.6- 8.9, *0.1*)	-3.3 (-8.4- 1.8, *0.3*)	8.7 (3.3- 14, *0.02*)
PVRi, Wood units*m^2	1.3 (-0.6- 3.2, *0.2*)	2.3 (0.6- 4, *0.009*)*	2.5 (-1.1- 6, *0.1*)	-1.9 (-9.3- 5.5, *0.6*)	3 (0.6- 5.4, *0.01*)*
Cardiac output, L/min	-0.04 (-0.5- 0.4, *0.8*)	0.5 (-0.3- 1.4, *0.2*)	0.8 (-0.4- 2, *0.2*)	1.4 (-1.8- 4.6, *0.4*)	-0.6 (-1.8- 0.7, *0.4*)*
Cardiac index, L/min*m^2	0.13 (-0.3- 0.6, *0.6*)	0.4 (-0.8- 1.5, *0.6*)	0.5 (-0.9- 1.8, *0.5*)	0.9 (-0.4- 2.2, *0.2*)	0.15 (-1.9- 1.6, 0.9)
6MWD, m	-38 (-76- 0.8, *0.05*)	59 (0.7- 117, *0.047*)*	29 (17- 42, 0.001)*	Inadequate n	Inadequate n
WHO-FC	-0.01 (-0.8-0.7, *0.9*)	-0.2 (-0.9-0.5, *0.6*)	0.4 (-3.3-4.1, *0.8*)	0.4 (-2.4-3.2, *0.8*)	1.13 (-0.9-3.2, *0.3*)

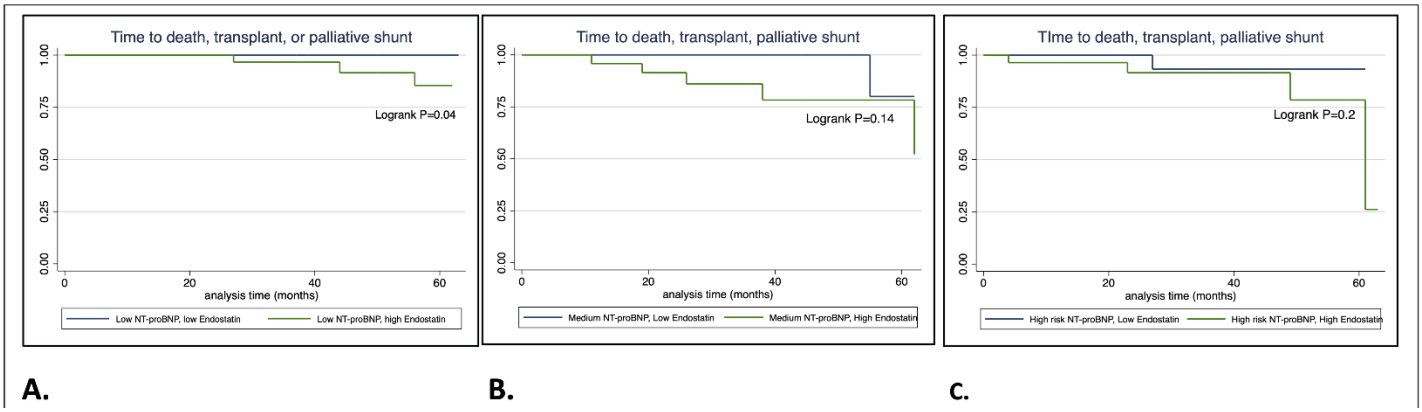
All data presented as regression coefficient (95% CI, p value). WHO-FC analyzed by ordinal logistic regression.
**p<0.05*
Cardiac output and cardiac index adjusted for only age and sex.
Definition of abbreviations: PAH: pulmonary arterial hypertension; PAHB: PAH biobank; CHC: Children's Hospital Colorado; APAH: associated PAH; APAH-CHD: Congenital heart disease associated PAH; IPAH: Idiopathic PAH; FPAH: familial PAH; ASD: atrial septal defect; VSD: ventricular septal defect

Table S5. Age- sex- and cardiac output- adjusted linear/logistic regressions of endostatin and continuous clinical variables in the Adult PAHB by CHD subtype.

	APAH-CHD All (n=185)	ASD (n=94)	VSD (n=42)	Complex CHD (N=49)
Age, years	5.9 (0.95-10.8, *0.02*)	10 (2-1-18, 0.014)	2.38 (-8.3-13, 0.7)	-1.8 (-15-11.5, 0.8)
BSA, m^2	-0.09 (-0.2—0.009, *0.03*)	-0.18 (-0.32—0.03, 0.02)	-0.07 (-0.25-0.11, 0.4)	0.14 (-0.13-0.4, 0.3)
mRAP, mmHg	2.1 (0.9- 3.3, *0.01*)*	2 (0.25- 3.7, *0.02*)	1.5 (-1.3- 4.4, *0.3*)	2.9 (0.5- 5.3, *0.02*)
mPAP, mmHg	-3.6 (-8.6- 1.5, 0.2)	-3.9 (-12- 3.8, *0.3*)	-5 (-17- 6.9, *0.5*)	2.5 (-6.8- 11, *0.6*)
PCWP, mmHg	0.9 (-0.4- 2, 0.2)	0.9 (-1.4- 3.3, *0.4*)	0.4 (-1.8- 2.6, *0.7*)	1.7 (-0.03- 3.5, *0.06*)
PVR, Wood units	-0.4 (-2.6- 1.8, *0.7*)	1.9 (-1.5- 5.2, 0.2)	0.6 (-4.2- 5.4, *0.8*)	1.5 (-2.5- 5.5, *0.4*)
PVRi, Wood units*m^2	4.8 (-0.9- 10.6, *0.1*)	12 (3.3- 21, *0.009*)*	5.9 (-6.3- 18, *0.3*)	-2.7 (-8.6- 3, *0.4*)
Cardiac output, L/min	-0.02 (-0.6- 0.6, *0.9*)	-0.5 (-1.4- 0.4, *0.3*)	-0.4 (-1.7- 0.8, 0.5)	1.3 (0.07- 2.5, *0.04*)
Cardiac index, L/min*m^2	0.2 (-0.5- 0.9, *0.6*)	0.6 (-0.6- 1.8, *0.3*)	0.02 (-0.9- 0.9, *0.9*)	0.6 (-0.6- 1.7, *0.4*)
6MWD, m	-47 (-88- -6, *0.02*)*	-43 (-104- 19, *0.1*)	-151 (-310- 8.6, *0.09*)	-49 (-125- 27, *0.2*)
WHO-FC	0.39 (-0.13-0.92, *0.14*)	0.59 (-0.32-1.5, 0.2)	0.72 (-0.47-1.9, 0.24)	0.18 (-1-1.4, 0.8)

All data presented as regression coefficient (95% CI, p value). WHO-FC analyzed by ordinal logistic regression.
**p<0.05*
Cardiac output and cardiac index adjusted for only age and sex.
Definition of abbreviations: PAH: pulmonary arterial hypertension; PAHB: PAH biobank; CHC: Children's Hospital Colorado; APAH: associated PAH; APAH-CHD: Congenital heart disease associated PAH; IPAH: Idiopathic PAH; FPAH: familial PAH; ASD: atrial septal defect; VSD: ventricular septal defect

Figure S1. Kaplan-Meier Curve demonstrating survival (death, transplant, Pott's shunt or atrial septostomy) by low, medium, and high NT-proBNP and low and high endostatin concentration in PAHB.



(A) Kaplan-Meier curve showing time to death, transplant, palliative shunt by low NT-proBNP (<200 pg/mL) and endostatin dichotomized by the mean (29.5 ng/mL). There was a significant difference in those with low NT-proBNP and low endostatin and those with low NT-proBNP and high endostatin (p=0.04). (B) Kaplan-Meier curve showing time to death, transplant, palliative shunt by medium NT-proBNP (200-400 pg/mL) and endostatin dichotomized by the mean (29.5 ng/mL). (C) Kaplan-Meier curve showing time to death, transplant, palliative shunt by high NT-proBNP (>400 pg/mL) and endostatin dichotomized by the mean (29.5 ng/mL).