Endostatin and ST2 are predictors of pulmonary hypertension disease course in infants.

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Abstract

Introduction: Pulmonary hypertension (PH) is a common comorbidity of cardiopulmonary disease. Endostatin, an inhibitor of angiogenesis, is elevated in neonates with lung disease. ST2 is a heart failure biomarker correlated with PH in adults. We hypothesized that these biomarkers may be useful in diagnosing PH and categorizing its severity in infants.

Methods: Endostatin, ST2, and NT-proBNP plasma concentrations from 26 infants with PH and 21 control infants without PH were correlated with echocardiographic and clinical features using regression models over time.

Results: Endostatin, ST2, and NT-proBNP concentrations were elevated in PH participants versus controls (p<0.0001). Endostatin was associated with right ventricular dysfunction (p=0.014), septal flattening (p=0.047), and pericardial effusion (p<0.0001). ST2 concentrations predicted right to left patent ductus arteriosus flow (p=0.009). NT-proBNP was not associated with PH features.

Conclusions: Endostatin and ST2 concentrations were associated with echocardiographic markers of worse PH in infants and may be better predictors than existing clinical standards.
Introduction:

Pulmonary hypertension (PH) is one of the most significant complications of infant lung disease with an extremely high burden of morbidity. With PH, risk of death is increased as high as 40% in the first year of life.(1) This type of PH is classified by the World Society for Pulmonary Hypertension (WSPH) as group 3 PH, specifically PH related to lung disease with impaired alveolar and vascular growth and maturation.(2) Development of alveolar-capillary units starts in utero, progresses rapidly in the first 18 months of life, and continues until about 8 years of age.(3) Conditions that injure developing alveoli and lung vasculature, such as neonatal infection, prematurity with development of bronchopulmonary dysplasia, and congenital anomalies like renal agenesis and congenital diaphragmatic hernia, may result in a unique forms of pulmonary hypertension that are distinct from PH in older children and adults.(4)

Current clinical practice involves screening infants at high risk due to prematurity or other insults for development of pulmonary hypertension. Screening typically is done by echocardiogram, usually with the addition of NT-proBNP (N-terminal prohormone of brain natriuretic peptide). Invasive hemodynamic testing by cardiac catheterization is the gold standard for diagnosis of PH and quantification of severity but is invasive and not without risk in infants. Unfortunately, echocardiographic measures are not as precise as cardiac catheterization at quantifying the severity of PH, although qualitative measures such as septal flattening and RV function may be adequate for diagnosing PH but without absolute quantification of pulmonary artery pressure and resistance.(5) NT-proBNP has shown good performance as a predictor of long term outcomes in neonatal and infant PH, but it is developmentally regulated and there is no current cutoff with diagnostic or prognostic value in pediatrics.(6, 7)

While the proximate cause of PH in neonates and infants varies, this group is similar in that they have immature alveoli with an accompanying immature pulmonary vascular bed.(2, 3) New biomarkers that are more specific to pulmonary vascular changes characteristic of infant PH are needed to improve diagnosis and prognostic ability in this sensitive population. Endostatin, a fragment of Collagen XVIII A, is expressed in the basement membrane of endothelial cells and is an angiogenic regulator. Endostatin has been associated with lung development and injury, and has been associated with infant lung disease and PH.(8, 9) Increased circulating levels of endostatin have also been shown to predict mortality in a prior adult study of PH. (16) ST2 is a peptide member of the interleukin 1 (IL-1) receptor family, found in both a membrane bound (ST2-L) and soluble form (sST2), with IL-33 acting as the functional ligand of both forms.(17) sST2 is a scavenger receptor of IL-33 which is a marker of cardiac stress and fibrosis.(10) Studies in older children and adults suggest that sST2 is more specifically associated with elevated pulmonary vascular pressure.(11) Thus, we hypothesized that endostatin and sST2 concentrations may serve to improve diagnosis and management of pulmonary hypertension in neonates and infants and give more insight into their changing physiology.

We evaluated endostatin, sST2, and NT-proBNP as markers of PH diagnosis, and clinical course in a group of infants with pulmonary hypertension secondary to perinatal
cardiopulmonary disease. To examine their utility, we assayed serial plasma samples from 26 infants with pulmonary hypertension (and 21 full-term controls) recruited from two tertiary care children’s hospitals and compared the resulting concentrations to echocardiographic and clinical data.

Methods:

Study Design

Study participants were recruited through the pulmonary hypertension cohort study, which is a part of the Institution-wide Prospective, Inception Multi-Cohorts Study of Individuals with Childhood-Onset Acute and Chronic Health Conditions (iPICS) at Johns Hopkins Children’s Center and the Johns Hopkins All Children’s Hospital. The iPICS enrollment protocol was approved by the Johns Hopkins University institutional review board. All participants were enrolled after giving informed written consent. Participants were enrolled at diagnosis of pulmonary hypertension and followed prospectively. Inclusion was based on diagnosis of pulmonary hypertension by echocardiogram and clinical assessment within 28 days of study enrollment. Clinical data, including echocardiographic findings, and a blood sample were collected at enrollment, then weekly while the participant was in the hospital for the first 4 weeks, and then at months 1, 2, 4 and 6 after diagnosis. If a participant was discharged and then re-hospitalized, data were collected at the time of readmission. As a pilot study, all available participants who met enrollment criteria were used. De-identified biological samples, clinical, and echocardiographic data were available for analysis for 26 participants (Table 1) with a total of 101 separate samples for analysis.

Control Participants—Healthy controls included children (N=21, age 1 month-6 months, Table 1) presenting for elective surgery at the Johns Hopkins Children’s Center. Control participants were evaluated by their pediatrician and the attending anesthesiologist prior to sample collection. Control participants had no chronic medical problems and carried only the diagnosis requiring surgery (supplementary table 1). Control participants with chronic pulmonary disease including asthma, as well as allergies, eczema, or any other condition requiring daily medication were excluded. Participants with cardiac or pulmonary disease were excluded. Samples obtained were discarded samples and were collected with Johns Hopkins IRB approval and waiver of informed consent.

Measures

Clinical variables: Clinical variables included gestational age, sex, age at the time of each sample and weight at the time of each sample. Medication therapy with pulmonary vasodilators was recorded and assessed by class; phosphodiesterase inhibitor (PDE5), endothelin receptor antagonist (ERA), and prostacyclin analog (PCA).

Echocardiographic variables: Echocardiography was performed and interpreted by the Johns Hopkins University pediatric echocardiography lab, which is an Intersocietal Accreditation Commission accredited lab (www.intersocietal.org/echo). All echocardiograms were performed as part of clinical care. All study echocardiograms and reports were then reviewed by an independent cardiologist with expertise in advanced...
cardiovascular imaging and pulmonary hypertension, for agreement with the clinical echocardiography reader.

All participants had an initial complete echo to evaluate all components of structure and function, followed by focused imaging for pulmonary hypertension according to the laboratory’s standard PH protocol. The protocol includes tricuspid regurgitation velocity, inter-ventricular septal curvature in systole (measured in parasternal short axis), early and end diastolic pulmonary insufficiency Doppler gradients, direction of flow and Doppler gradient across a patent ductus arteriosus (PDA), patent foramen ovale (PFO), or across a ventricular septal defect (VSD). Right atrial pressure was assumed to be 5mmHg unless a concurrent central venous pressure was recorded in the study. All Doppler gradients were converted to pressures using the modified Bernoulli equation \(4V^2 +5\text{mmHg}\). Pulmonary hypertension was defined as an estimated PA pressure of at least \(\frac{1}{2}\) systemic pressure based on any/combination of tricuspid regurgitation (TR) velocity, PDA gradient, VSD gradient, or significant septal flattening. A bidirectional shunt across a PDA or VSD was considered to mean equivalent pressures between vessels/chambers. A right to left shunt across a PDA or VSD was considered to mean a higher than systemic pulmonary artery pressure, or higher than systemic right ventricular pressure.

Echocardiographic variables included measures of right ventricular function (RV function and tricuspid annular plane systolic excursion, TAPSE), right ventricular hypertension (tricuspid regurgitation, TR velocity, septal flattening, ASD, VSD, PDA and PFO flow direction), and presence of a pericardial effusion. Echocardiographic findings of flow direction (across PDA, ASD, VSD, PFO) were assessed based on Doppler interrogation of the flow on echo. Qualitative measures of RV hypertension including septal flattening, and qualitative assessment of RV function were assessed as either present or not present by the interpreting cardiologist. Direction of flow across a PDA was assessed as sub systemic (left to right) or systemic or greater (bidirectional or right to left). Direction of flow across a VSD or ASD was assessed as either pressure lower than LV or LA respectively (left to right shunt) or pressure equal or greater than LV or LA respectively (bidirectional/right to left shunt).

**Biomarkers**

A multiplex electrochemiluminescent immunosorbent assay was developed to measure endostatin, sST2, and NT-proBNP simultaneously using robotically spotted capture antibodies on the 96-well plate Meso Scale Discovery platform (Meso Scale Discovery [MSD], Gaithersburg, MD). Capture antibody-spotted plates were blocked with 5% BSA-PBS complemented with .05% TWEEN (PBS-T) and incubated at room temperature on an orbital shaker (500 rpm) for 60 minutes. Calibrators for endostatin (R&D 841457), sST2 (R&D 840760), and NT-proBNP (MSD C01XX-1) were produced using commercially provided diluent (MSD R51BB-3) with a concentration range of 16000–72.7 pg/mL, 8300–2.03 pg/mL, 2000–1.12 pg/mL, respectively. Samples were diluted 15x in commercially provided diluent (MSD R51BB-3). The detection antibody cocktail for endostatin (R&D 841456), sST2 (R&D 840354), and NT-proBNP (MSD D21JK-1) was prepared in
commercially provided diluent (MSD R51BA-5) and supplemented with 0.5 μg/mL SA-Sulfo-tag (MSD, R32AD-5). Finally, after incubation and washing, 150 μl of 1X commercially provided read buffer (MSD R92TC-1) was applied to the plate and promptly read in an MSD Sector Imager 2400. The limits of detection for the endostatin, sST2, and NT-proBNP assays were 0.073ng/mL, 0.002 ng/mL, and 20 pg/ml, respectively. All assays were completed in the same lab, by the same technician. All assays were blinded for clinical outcomes and only unblinded for statistical analysis.

**Statistical Analysis:** Summary statistics were generated for the biomarkers including endostatin, sST2, and NT-proBNP concentration at enrollment and statistical comparisons between PH participants and control participants were conducted using Wilcoxon rank sum tests. Demographic, clinical, and biomarker data are presented as median and IQR, or median, percent, and range as appropriate. Receiver operator characteristic curves (ROC) were generated to determine the sensitivity and specificity of endostatin, sST2, and NT-proBNP at differentiating PH from healthy controls. Optimal cut points were calculated by Youden analysis. Biomarker data were non-normally distributed, and log transformed for multivariate linear and logistic regression analysis. We conducted univariate linear regression at enrollment for main hypothesis testing. Each biomarker was separately defined as the independent variables and clinical variables were defined as the dependent variables; this was performed to assess whether there was an association between clinical variables and biomarkers. The effect of the biomarker on the clinical outcomes over time were then assessed in a multivariable linear or logistic regression model. Regression analyses were adjusted for gestational age, gender, and age at the time of sample collection. All regressions analyses also accounted for the longitudinal, nested nature of the data, i.e. multiple samples per participants over time, using mixed linear and logistic models. Statistical analyses were performed using Stata 15.1 (StataCorp, LLC, College Station, TX) and p values < 0.05 were considered statistically significant.

**Results**

**Participant Demographics:**

All PH participants (n=26) were diagnosed with WSPH group 3 PH based on echocardiographic assessment (RV pressure by TR velocity, PDA gradient, VSD gradient, septal flattening) by the reading cardiologist within 28 days of enrollment. All had associated lung disease, with a mean length of follow up of 3 visits over 97.3 days. There were 21 control participants available for analysis. The demographics of the PH participants and controls are shown in Table 1. Presenting complaints for the control cohort is shown in supplementary table 1. In the PH cohort, 39% of the participants were full term gestation (>37 weeks) and 61% preterm gestation (<37 weeks). The median gestational age was 33 weeks for the PH cohort, with a median of 38.5 weeks in full term participants and 27 weeks in premature participants. The median age at enrollment of PH participants was 33.5 days, with a median of 67.5 days in the full-term participants and 29.5 days in the premature participants, which was younger than the median recruitment age of the control cohort (180 days; p=0.013). Half of the PH cohort was female, which did not differ from the control cohort (p=0.63). Within the PH participants, 46% were diagnosed with bronchopulmonary
dysplasia, 11.5% congenital diaphragmatic hernia, 7.6% congenital heart disease in addition to lung disease, and 7.6% with Trisomy 21. The PH participants had a median tricuspid regurgitation velocity of 2.8m/s, with a median estimated RV systolic pressure of 36mmHg.

**Serum Endostatin, sST2 and NT-proBNP in Pediatric PH:**

The results of the serum endostatin, sST2, and NT-proBNP concentrations are detailed in Table 1 and Figure 1 for both the PH and healthy control (median and IQR) cohorts at enrollment. Serum concentrations for endostatin, sST2, and NT-proBNP were all elevated in the PH group compared with healthy controls (all \(p<0.001\)). Hypothesis testing was performed using univariate linear regression at enrollment with the biomarker as the outcome variable. The concentrations of endostatin, sST2, and NT-proBNP was associated with presence of PH (\(p<0.0001\) for all three biomarkers) (Table 2), but the biomarkers were not associated with gender, age, gestational age, or weight. In the presence of pulmonary hypertension, endostatin concentration was 71.6 ng/mL higher, sST2 was 20.2ng/mL higher, and NT-proBNP was 4.74 ng/mL higher than in the controls. There was no significant change in concentration of endostatin, sST2, and NT-proBNP based on gender, age, gestational age, or weight (Table 2). Within the PH cohort, endostatin was higher in the full-term participants compared with the preterm participants (Table 1, \(p=0.006\)). sST2 and NT-proBNP were not significantly different in the full term versus preterm infants within the PH cohort (Table 1, \(p=0.13\) and \(p=0.51\), respectively).

**Endostatin, sST2, and NT-proBNP concentration discriminates PH from controls:**

To assess whether endostatin, sST2, and NT-proBNP discriminated PH from controls, we performed ROC analysis (Figure 2). Optimal cut points for each biomarker calculated based on the maximum Youden index. Endostatin differentiated PH from controls with an AUC of 0.99 (95% CI 0.98–1, \(p<0.0001\)). At a cut point of 57.4 ng/mL, endostatin had a sensitivity of 96.8% and specificity of 100% in differentiating PH from healthy controls. This cutoff had a positive predictive value of 95% for identifying PH and a negative predictive value of 84%. sST2 yielded an AUC of 0.79 (95% CI 0.66–0.91, \(p<0.0001\)). At a cut point of 3.14 ng/mL, sST2 had a sensitivity of 75.3% and specificity of 81% in discriminating PH from healthy controls. This cutoff for sST2 had a positive predictive value for PH of 95.9% and negative predictive value of 36%. NT-proBNP was similar to sST2 with an AUC of 0.82 (95% CI 0.711–0.93, \(p<0.0001\)). At a cut point of 0.262 ng/mL, NT-proBNP had a sensitivity of 76% and a specificity of 81% in discriminating PH from healthy controls. This cutoff had a positive predictive value for identifying PH of 95% and a negative predictive value of 37%.

**Endostatin concentration is associated with echocardiographic measures of right ventricular dysfunction:**

Endostatin, sST2, and NT-proBNP were evaluated against echocardiographic measures of cardiac function including TAPSE, overall RV dysfunction (Figure 3A), left ventricular ejection fraction, presence of a pericardial effusion (Figure 3B) and presence of a VSD (Figure 3C) at each visit. Regression results are shown in Table 3A with specific data on major echocardiographic variables. Endostatin, sST2, and NT-proBNP concentrations were not associated with TAPSE or left ventricular ejection fraction. Increased endostatin was
associated with presence of right ventricular dysfunction; with a 10-fold increase (log-increase) in endostatin the adjusted odds ratio of RV dysfunction was 27.15 (95% CI 1.87–374, p=0.014). Higher endostatin was also associated with the presence of a pericardial effusion; for every log increase in endostatin concentration, the odds ratio of pericardial effusion was 14.5 (95% CI 3.7–56.3, p<0.0001). Endostatin and sST2 were associated with less risk for the presence of a VSD (adjusted OR 0.008 and 0.09, p= 0.029 and 0.024 respectively). sST2 and NT-proBNP were not associated with presence of RV dysfunction (p=0.14 and p=0.67 respectively).

**sST2 and Endostatin concentration are associated with echocardiographic measures of pulmonary hypertension:**

Endostatin, sST2, and NT-proBNP were evaluated against echocardiographic variables of pulmonary hypertension, including TR velocity, tricuspid regurgitation peak gradient, presence of septal flattening, and direction of flow across a PFO or PDA at each visit (Table 3B, Figure 3D-3E). Endostatin, sST2, and NT-proBNP were not associated with quantitative measures of pulmonary hypertension, specifically tricuspid regurgitation. Endostatin was associated with flattening of the interventricular septum with an adjusted odds ratio of 7.76 (95% CI 1.03–58.5, p=0.047); thus, for every log (10-fold) increase in endostatin concentration, the odds ratio of septal flattening was increased to 7.76. sST2 was associated with evidence of PH based on direction of blood flow across a PDA with an adjusted odds ratio of 159 (95% CI 3.6–7061, p=0.009) for a bidirectional or right to left shunt across the PDA, suggesting systemic or supra-systemic PA pressure. Thus for every log increase in sST2, the odds ratio of having systemic or supra-systemic PA pressure was 159. NT-proBNP was not associated with presence of septal flattening (p=0.086), or with bidirectional or right to left PDA flow (p=0.27).

**Endostatin, sST2, and NT-proBNP were not associated with type or duration of medical therapy:**

There was no association between endostatin, sST2, or NT-proBNP concentration and the number of PH medication classes (Table 3C) prescribed. There was no association with endostatin, sST2, or NT-proBNP and use of an endothelin receptor antagonist (p=0.19, p=0.56, and p=0.68 respectively), a phosphodiesterase inhibitor (p=0.77, p=0.48, and p=0.82 respectively) or a prostacyclin analog (p=0.26, p=0.32, and p=0.41 respectively).

**Discussion:**

Diagnosis and management of infants with pulmonary hypertension present a unique challenge to clinicians. Current diagnosis and management do not consider the pathogenesis of PH in an infant with an immature but rapidly growing pulmonary vascular bed, and shifting right ventricular hemodynamics associated with changing physiology. In this study of PH associated with infant lung disease, we found that endostatin and sST2 were elevated and consistently associated with echocardiographic markers of PH and right ventricular dysfunction, while NT-proBNP did not perform as well. The sensitivity and specificity of endostatin and sST2 suggest that they could be used to diagnose pulmonary hypertension, which may be especially helpful in infants where invasive diagnostics are high risk and
echocardiograms are not as sensitive. More importantly, endostatin and sST2 were also associated with other clinical markers of elevated pulmonary artery pressure, and with right ventricular dysfunction over multiple time points. This has the potential as a non-invasive marker to follow progression of disease and guide therapy in infants. Using biomarkers to guide therapy would need to be evaluated in a larger, prospective study. Notably, increased endostatin was associated with a lower odds of having a VSD, in contrast to the increased risk of RV dysfunction. This may be because the presence of a VSD offers an alternative outflow in the setting of pulmonary hypertension, thus preventing ventricular dysfunction; however, with only 2 participants with a VSD (see Table 1), we could not make any definitive conclusions about this relationship. Endostatin and sST2 may be more informative markers of ventricular function and pulmonary hypertension in infants with WSPH group 3 pulmonary hypertension (related to lung disease).

Endostatin is an endogenous angiogenic inhibitor that is a proteolytic fragment of the C-terminal domain of Collagen XVIIIA. Collagen XVIIIA is ubiquitously expressed in vascular and endothelial basement membrane structures; the C-terminal endostatin domain in particular is localized across the elastic fibers of large arteries. Endostatin inhibits angiogenesis by inhibiting endothelial cell proliferation and migration, as well as inducing endothelial cell apoptosis. It has multisystem effects, including developmental effects, as well as a role in injury and repair and has been shown to inhibit repair of lung epithelium in pulmonary fibrosis. Angiogenesis is essential for development of the lung, with VEGF shown to be important for alveolarization and the accompanying vascular bed; endostatin, as an angiogenic inhibitor, including an inhibitor of VEGF, has been found to be increased in the lungs of preterm infants, and those with bronchopulmonary dysplasia. A genetic variant of Collagen 18a1, the precursor of endostatin, which caused increased concentrations of endostatin, was shown to predict mortality in a prior study of PH in adults. In this study, endostatin was both sensitive and specific for PH, and was associated with markers of both PH and RV dysfunction.

Soluble sST2 is produced in multiple tissues, including endothelial cells, and cardiomyocytes. It is a biomarker of cardiac stress and is significantly increased in the setting of cardiac stretch. Animal studies have demonstrated upregulation of HIF1-α and VEGF in the setting of increased sST2; sST2 knockout mice were conversely protected from development of hypoxic pulmonary hypertension. In studies of sST2 as a biomarker of heart failure in adults and older children, sST2 was consistently correlated with RV systolic pressure, tricuspid regurgitation, and right ventricular function. In studies of pediatric patients with WSPH group 1 PH, sST2 was associated with worse NYHA functional class, as well as with mortality. sST2/IL-33 is further increased in animal and in vitro models of hypoxic pulmonary hypertension with subsequent downstream activation of the HIF/VEGF system causing growth and hypertrophy of pulmonary vascular endothelial cells. In this study, sST2 outperformed NT-proBNP at assessing increased pulmonary artery pressure, possibly because it is more sensitive in this population, or because it is not subject to developmental regulation.
This study is limited by a small sample size limiting the ability to assess the association of biomarkers with clinical findings. The diagnosis and management of pulmonary hypertension in these participants also relied on echocardiography, rather than cardiac catheterization, which is a less sensitive and less accurate measure of pulmonary hypertension. The control cohort used in this study were healthy children, demonstrating that the markers are different in PH compared to healthy controls, but not compared to a control cohort with lung disease, but not PH. Future studies should evaluate endostatin and sST2 in a larger cohort of infants with lung disease, both with and without pulmonary hypertension to better evaluate the sensitivity and specificity of the biomarkers in discriminating lung disease associated PH from other disease states. It should also be noted that neither endostatin, sST2, nor NT-proBNP correlated with prescribed medical therapies for PH, which may reflect the small sample size of the study or potentially the need for ongoing discovery studies to identify improved markers to follow disease.

In this study, endostatin and sST2 performed as well as, or better, than NT-proBNP in differentiating pulmonary hypertension from controls. Given the potential role of endostatin in alveolar capillary development, increasing endostatin concentrations may be an early marker of pulmonary hypertension in this unique population. sST2 may be a more sensitive and specific marker of cardiopulmonary stress in infants. Both endostatin and sST2 should be further evaluated as biomarkers to follow infants with WSPH group 3 pulmonary hypertension.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations list:**

- **PAH** pulmonar artery hypertension
- **BPD** Bronchopulmonary Dysplasia
- **CDH** Congenital Diaphragmatic Hernia
- **AUC** area under the curve
- **WSPH** World Symposium on Pulmonary Hypertension
- **ELISA** enzyme-linked immunosorbent assay
- **IQR** interquartile range
- **TAPSE** Tricuspid Annular Plane Systolic Excursion
PDA  Patent Ductus Arteriosus
PFO  Patent Foramen Ovale
VSD  Ventricular Septal Defect
ASD  Atrial Septal Defect
RV  Right Ventricle
PDE  Phosphodiesterase Inhibitor
ERA  Endothelin Receptor Antagonist
PCA  Prostacyclin Analog

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Figure 1. Endostatin, ST2, and NT-ProBNP in pulmonary hypertension versus controls (ng/mL, median and IQR) at enrollment.

P-Values represent difference between each biomarker concentration in PH and controls. Significant difference (by rank sum test) between PH and controls for Endostatin (p<0.0001), ST2 (p=0.0003) and NT-proBNP (p=0.0001).
Figure 2. ROC curve of Endostatin, ST2, and NT-ProBNP in pulmonary hypertension vs. controls

Optimal cutoff of each biomarker based on the maximum Youden index. For Endostatin, a cut point of 57.3 ng/mL was 96.8% sensitive and 99% specific for identifying PH versus controls. For ST2, a cut point of 3.14 ng/mL was 75.3% sensitive and 81.2% specific for PH versus controls. For NT-proBNP, a cut point of 0.262 ng/mL was 76.3% sensitive and 81.2% specific for PH versus controls.
Figure 3. Biomarker concentrations in PH subjects by echocardiographic finding (adjusted over multiple visits).
Biomarker concentration (ng/mL, median and IQR) for the presence/absence of each echocardiographic finding.
A. Endostatin, ST2, and NT-proBNP in normal right ventricle versus right ventricular dysfunction.
B. Endostatin, ST2, and NT-proBNP in a pericardial effusion versus no effusion.
C. Endostatin, ST2, and NT-proBNP in a VSD versus no VSD.
D. Endostatin, ST2, and NT-proBNP in a round interventricular septum versus flattened interventricular septum.
E. Endostatin, ST2, and NT-proBNP in left to right or bidirectional/right to left PDA flow.
Table 1.

|                              | PH Cohort (n=26) | Control Cohort (n=21) | P value | PH Full Term Sub-cohort (n=10) | PH Preterm Sub-cohort (n=16) | P value |
|------------------------------|------------------|-----------------------|---------|-------------------------------|-----------------------------|---------|
| Sex (% female)               | 50%              | 42%                   | 0.63    | 40%                           | 56%                         | 0.24    |
| Median age at enrollment (days) | 33.5 (14.7, 142) | 180 (109, 292)       | 0.013   | 67.5 (13.5, 718)              | 29.5 (15, 121)              | 0.47    |
| Mean Duration of follow-up, days (SEM) | 97.3 (173)       | 100 (128)            | 0.013   | 95 (204)                      | 0.94                        |
| Mean number of visits (SEM)   | 3 (1.8)          | 3.3 (2)              | 0.21    | 2.8 (1.7)                     | 0.21                        |
| Median weight at enrollment (kg)  | 3.5 (2.8, 6)   | 3.3 (2.125, 3.55)    | 0.13    | 3.89 (3.2, 6.1)               | 0.13                        |
| Median gestational age at birth (weeks) | 33 (25, 38)  | 38.5 (38, 39)        | <0.001  | 27 (24, 31)                   | <0.001                      |
| Median TR Velocity (m/s)      | 2.8 (2.5, 3.7)  | 3.2 (2.5, 3.9)       | 0.86    | 2.8 (2.3, 3.6)                | 0.86                        |
| TR peak gradient (mmHg)       | 36 (25, 72)     | 60 (26, 86)          | 0.55    | 32 (22, 54)                   | 0.55                        |
| Bronchopulmonary Dysplasia (% yes) | 46%            | 0%                   | <0.0001 | 75%                           | <0.0001                     |
| Congenital Diaphragmatic Hernia (% yes) | 11.5%          | 30%                  | 0.035   | 0%                            | 0.035                       |
| Congenital Heart Disease (% yes) | 7.6%            | 20%                  | 0.01    | 0%                            | 0.01                        |
| Trisomy 21 (% yes)            | 7.6%             | 20%                  | 0.24    | 0%                            | 0.24                        |
| Endostatin (ng/mL)            | 107.6 (89.3, 117) | 33.7 (27.1, 35.6) | <0.0001 | 117.5 (105.9, 143.8)         | 93.4 (74.4, 112.2)         | 0.006   |
| ST-2 (ng/mL)                  | 7.2 (3.6, 22.1) | 1.9 (1.8, 2.9)       | 0.0003  | 13.2 (6.2, 50.4)              | 3.3 (2.2, 12.5)            | 0.13    |
| NT-ProBNP (ng/mL)             | 2.037 (0.498, 6.22) | 0.103 (0.067, 0.245) | 0.0001  | 1.899 (0.433, 6.29)          | 0.587 (0.197, 2.28)        | 0.51    |

Descriptive statistics are reported at enrollment.

All regressions were nested by subject to account for multiple data points from the same individual.
Table 2.

Endostatin, ST2, NT-ProBNP concentration based on demographic variables at enrollment

| Univariate Linear Regressions | Endostatin (ng/mL) | ST2 (ng/mL) | NT-ProBNP (ng/mL) |
|-------------------------------|-------------------|-------------|-------------------|
| Pulmonary Hypertension (1=yes, 0=no) | 71.6 (58.8, 84.7) | 20.2 (1.1, 39) | 4.74 (0.989, 9.38) |
| Sex (1=female, 0=male) | 2.5 (−23, 28) | 3.75 (−15, 23) | −0.170 (−4.94, 4.59) |
| Age at sample (years) | −0.002 (−0.018, 0.014) | −0.003 (−0.015, 0.0009) | −0.01 (−4.94, 4.59) |
| Gestational Age (weeks) | 0.15 (−0.02, 3.1) | 0.18 (−2.4, 2.7) | 0.123 (−0.5, 0.75) |
| Weight (kg) | −0.48 (−2.3, 1.4) | 0.54 (−2.2, 3.3) | −0.224 (−0.9, 0.45) |

Clinical variable is independent, and biomarker is dependent variable. Thus, in the presence of pulmonary hypertension, Endostatin increased by 71.6 ng/mL, ST2 increased by 20.2 ng/mL, and NT-proBNP increased by 4.74 ng/mL.
Table 3A.

Endostatin, ST-2 and NT-ProBNP Associations with Clinical Measures of Ventricular Function longitudinally

| Ventricular Function                      | (log) Endostatin   |   | (log) ST-2       |   | (log) NT-ProBNP |   |
|------------------------------------------|--------------------|---|-----------------|---|-----------------|---|
| Adjusted Linear Regressions              | Coefficient (95% CI) | P value | Coefficient (95% CI) | P value | Coefficient (95% CI) | P value |
| TAPSE (cm)                               | -0.14 (-0.49, 0.22) | 0.44 | 0.0075 (-0.073, 0.08) | 0.85 | 0.029 (-0.051, 0.11) | 0.46 |
| Left Ventricle Ejection Fraction (%)     | 32.1 (-130, 194)   | 0.69 | 29.1 (-6.99, 65.2) | 0.11 | 10.5 (-18.8, 39.7) | 0.47 |
| Adjusted Logistic Regressions            | Odds Ratio (95% CI) | P value | Odds Ratio (95% CI) | P value | Odds Ratio (95% CI) | P value |
| RV Dysfunction (Yes or no)               | 27.15 (1.87, 374)  | <0.014 | 1.36 (0.9, 2.05)  | 0.14 | 1.1 (0.79, 1.4)   | 0.67 |
| Pericardial Effusion (Yes or no)         | 14.5 (3.7, 56.3)   | <0.0001 | 1.07 (0.74, 1.55) | 0.73 | 0.97 (0.66, 1.45) | 0.89 |
| Ventricular Septal Defect (Yes or no)    | 0.008 (0.0001, 0.61) | 0.029 | 0.09 (0.012, 0.73) | 0.024 | 0.81 (0.26, 2.5) | 0.72 |

All regressions were adjusted for sex, age at time of sample, and gestational age.

All regressions were nested by subject to account for multiple data points from the same individual over multiple visits.

All biomarkers were log transformed for non-normality. Thus, coefficients represent change in clinical variable, or increased odds ratio of clinical variable for every log (10-fold) increase in biomarker concentration.
Table 3B.
Endostatin, ST-2 and NT-ProBNP Associations with Clinical Measures of Pulmonary Artery Pressure longitudinally

| Pulmonary Artery Pressure                          | (log) Endostatin            | (log) ST-2                   | (log) NT-ProBNP              |
|---------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Adjusted Linear Regressions**                   | Coefficient (95% CI)        | P value                     | Coefficient (95% CI)        | P value                     | Coefficient (95% CI)        | P value                     |
| Tricuspid Regurgitation Peak Gradient (mmHg)      | 243.8 (–66, 553)            | 0.12                        | −63.5 (–135, 8.1)           | 0.08                        | −50.9 (–177, 76)            | 0.41                        |

| Adjusted Logistic Regressions                     | Odds Ratio (95% CI)         | P value                     | Odds Ratio (95% CI)         | P value                     | Odds Ratio (95% CI)         | P value                     |
| Septal Flattening (Yes or no)                     | 7.76 (1.03, 58.5)           | 0.047                       | 1.05 (0.75, 1.4)            | 0.75                        | 1.26 (0.97, 1.65)           | 0.086                       |
| PDA flow direction (Left to right or Bidirectional/Right to left) | 1.08 (0.00015, 7986)     | 0.99                        | 159 (3.6, 761.5)           | 0.009                       | 1.37 (0.78, 2.44)           | 0.27                        |
| Patent Foramen Ovale Flow Direction (Left to right or Bidirectional/Right to left) | 6.14 (0.22, 172)          | 0.29                        | 0.93 (0.32, 2.73)          | 0.9                         | 1.2 (0.366, 3.94)           | 0.76                        |

All regressions were adjusted for sex, age at time of sample, and gestational age.

All regressions were nested by subject to account for multiple data points from the same individual over multiple visits.

All biomarkers were log transformed for non-normality. Thus, coefficients represent change in clinical variable, or increased odds ratio of clinical variable for every log (10-fold) increase in biomarker concentration.
Table 3C.

Endostatin, ST-2 and NT-ProBNP Associations with Medical Therapy longitudinally

| Medical Therapy                        | (log) Endostatin | (log) ST-2       | (log) NT-ProBNP  |
|----------------------------------------|------------------|-----------------|-----------------|
| **Adjusted Linear Regressions**        |                  |                 |                 |
| Total medication Number                | −0.71 (−2.75, 1.3) | 0.45            | 0.23 (−0.25, 0.72) | 0.33 | −0.12 (−0.41, 0.17) | 0.42 |
| Length of medical therapy (Days)       | 11.3 (−25, 47.7)  | 0.53            | 1.97 (−5.4, 9.3) | 0.58 | 1.24 (−4.25, 6.73) | 0.65 |
| **Adjusted Logistic Regressions**      |                  |                 |                 |
| Phosphodiesterase Inhibitor (Yes or no)| 1.31 (0.21, 8.1)  | 0.77            | 1.15 (0.78, 1.7) | 0.48 | 0.97 (0.75, 1.25) | 0.82 |
| Endothelin Receptor Antagonist (Yes or no) | 0.2 (0.018, 2.3) | 0.19            | 1.23 (0.61, 2.5) | 0.56 | 0.91 (0.58, 1.42) | 0.68 |
| Prostanoid Analog (Yes or no)           | 0.33 (0.05, 2.3)  | 0.26            | 1.3 (0.76, 2.29) | 0.32 | 0.87 (0.61, 1.22) | 0.41 |

All regressions were adjusted for sex, age at time of sample, and gestational age.

All regressions were nested by subject to account for multiple data points from the same individual over multiple visits.

All biomarkers were log transformed for non-normality. Thus, coefficients represent change in clinical variable, or increased odds ratio of clinical variable for every log (10-fold) increase in biomarker concentration.