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Pediatric Pulmonary Hypertension: Insulin Like Growth Factor Binding Protein 2 is a novel marker associated with disease severity and survival

Megan Griffiths[1], **Jun Yang**[1], **Melanie Nies**[1], **Dhananjay Vaidya**[2], **Stephanie Brandal**[1], **Monica Williams**[3], **Elizabeth C. Matsui**[4], **Torie Grant**[5], **Rachel Damico**[3], **Dunbar Ivy**[6], **Eric D. Austin**[7], **William C. Nichols**[8], **Michael W. Pauciulo**[8], **Katie Lutz**[8], **Erika B. Rosenzweig**[9], **Russel Hirsch**[10], **Delphine Yung**[11], **Allen D. Everett**[1]

[1.]Division of Pediatric Cardiology, Department of Pediatrics, Johns Hopkins University, Baltimore, MD

[2.]Department of Internal Medicine, Johns Hopkins University, Baltimore, MD

[3.]Department of Anesthesia and Critical Care Medicine, Johns Hopkins University, Baltimore, MD

[4.]Departments of Population Health, Department of Pediatrics, Dell Medical School, University of Texas, Austin, TX

[5.]Division of Allergy and Immunology, Department of Pediatrics, Johns Hopkins University, Baltimore, MD

[6.]Department of Pediatric Cardiology, Children's Hospital Colorado, Denver, CO

[7.]Division of Allergy, Immunology, and Pulmonary Medicine, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN

[8.]Division of Human Genetics, Cincinnati Children's Hospital Medical Center; Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio

[9.]Division of Pediatric Cardiology; Department of Pediatrics; Columbia University, New York City, NY

[10.]Division of Pediatric Cardiology; Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio

[11.]Division of Pediatric Cardiology; Department of Pediatrics, University of Washington, Seattle, WA

[*]Correspondence and requests for reprints should be addressed to Allen D. Everett, M.D. Division of Pediatric Cardiology, Department of Pediatrics, Johns Hopkins University, 1800 Orleans St. Room M2303, Baltimore, MD 21287. Phone: 410-955-5987; aeveret3@jhmi.edu.

Author Contributions: M.G., J.Y., M.K.N. and A.D.E planned the project, analyzed the data and wrote the manuscript; M.G., S.B. and J.Y. performed the experiments and interpreted the results; M.G., R.D., J.Y. performed statistical analysis; M.K.N., M.W., E.M., T.G., R.D., D.I., E D A, W.C.N, M.W.P, K.L., E.B.R., R.H., D.Y., and A.D.E recruited subjects and performed research; all authors reviewed, revised and approved the manuscript for submission.

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Abstract

Background: Insulin like growth factors (IGFs), and their binding proteins (IGFBPs), play a significant role in cardiovascular function and may influence the pathobiology of PAH. We determined the diagnostic and prognostic value of IGF1 and IGFBP2 in pediatric PAH.

Methods: Serum was analyzed by ELISA for IGF1, and IGFBP2 in pediatric PAH subjects from the NHLBI PAH Biobank (PAHB, n=175), and a cohort of asthmatic subjects (n=46, age 0-21) as a chronic pediatric pulmonary disease control. Biomarkers were analyzed with demographic, and clinical variables for PAH severity.

Results: Serum IGF1 was significantly lower in PAH compared to controls, while IGFBP2 was elevated in PAH subjects compared to controls. In the PAHB, IGF1 was negatively associated with mPAP and PVR, while IGFBP2 was positively associated with PVR and negatively associated with cardiac output, and 6 minute-walk-distance. Higher IGFBP2 levels were associated with use of prostacyclin therapy. IGFBP2 was associated with death, transplant, or palliative shunt with a Cox proportional hazard ratio of 8.8 ($p<0.001.$), but not IGF1 ($p=0.13$).

Conclusion: Circulating IGFBP2 is a novel marker for pediatric PAH which is associated with worse functional status, and survival. IGF axis dysregulation may be an important mechanistic target in pediatric pulmonary arterial hypertension.

Introduction:

Pulmonary arterial hypertension (PAH) in children is a progressive and almost uniformly fatal disease characterized by sustained elevation of pulmonary arterial pressures and death from right ventricular failure.(1) Pediatric pulmonary arterial hypertension (World Symposium on Pulmonary Hypertension (WSPH) group 1) is a heterogeneous disease which may be idiopathic (IPAH), familial or heritable (FPAH/HPAH), or associated with congenital heart disease (APAH-CHD), infection, portal hypertension, and pulmonary venous/capillary diseases (PVOD/PCH)(2). The distribution of disease associations is different in children with a higher incidence of IPAH, HPAH, and congenital heart disease rather than connective tissue disease, or infection.(1) (3, 4)

The growth and metabolic regulation of the pulmonary vasculature and right ventricle in PAH is incompletely understood, but is characterized by perivascular infiltration of inflammatory cells, and loss of small arteries and capillary beds.(5) Insulin like growth factors (IGF1/IGF2) are essential in the growth of endothelium and smooth muscle, development of vascular networks, and cardiomyocyte development and function.(6) IGF actions are mediated by the IGF1/IGF2 receptors; in turn, IGF receptor interactions are modulated by a family of seven high-affinity IGF binding proteins (IGFBP1-7)(7). While IGF1 is the primary mediator, most IGF1 is bound to and stabilized by the IGF binding proteins (IGFBPs) which facilitate transport and interaction of IGF with the IGF receptor, and act as a reservoir for IGF1 in the circulation.(8) Of the IGF binding proteins, IGFBP2 has non-IGF mediated functions including translocation to the nucleus, affecting gene expression, with cell growth and developmental effects.(9, 10)

IGFBP2 has also been implicated in cardiopulmonary disease. Circulating IGFBP2 was associated with pulmonary fibrosis, and with a treatment response.(11) IGFBP2 has also shown promise differentiating heart failure severity, with additional diagnostic benefit above standard biomarkers.(12) The role of IGFBP2 is poorly understood in PAH, but its IGF-independent actions are well-described in angiogenesis. In diseases such as cancer, IGFBP2 has been shown to have a pro-angiogenic and mitogenic effect contributing to disordered vascular growth, a mechanism that may have relevance in PAH.(9)

Elevation of circulating IGFBP2 was identified in a proteomic analysis of adult PAH subjects against controls.(13) Given the activity of IGFBP2 in abnormal vascular growth, the role of IGF1 in cardiomyocyte function, and the elevated IGFBP2 in adult PAH subjects, this study examines the relationship of IGFBP2 and IGF1 as predictors of pediatric PAH severity.

Methods:

In this multicenter cross-sectional study of pediatric PAH, we determined and analyzed IGF1, and IGFBP2 concentrations from a cohort of pediatric subjects with WSPH group 1 PAH and a cohort of children with asthma as chronic lung disease controls.

Study Cohorts:

NHLBI PAH Biobank enrollment protocol and informed consent was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. Control samples from Johns Hopkins Hospital were collected after informed consent, or with waiver of consent, by Johns Hopkins investigators. All cohorts have been approved by the Johns Hopkins University Institutional Review Board.

Pulmonary Hypertension Enrollees:

NHLBI PAH Biobank: The PAH Biobank (PAHB) is a NHLBI (HL105333) funded resource of biological samples, genetic and clinical data for the PAH research community, with 5 pediatric enrolling centers (www.pahbiobank.org). De-identified biological samples and clinical data on enrollees (n=175, < 21 years old, Table 1) were available for analysis. The PAHB collects subject clinical parameters at enrollment, demographics and comorbidities, NYHA/WSPH functional class, 6-minute walk distance (6MWD), drug therapy and right heart catheterization data. A subset of the PAHB cohort had right heart catheterizations within 6 months of enrollment (n=29) and were used for invasive cardiac hemodynamic analysis. Mortality, transplant, and palliative shunt (Pott's shunt or atrial septostomy) was collected prospectively and analyzed for the entire cohort.

Pediatric Asthma Subjects: A cohort of asthmatic pediatric subjects was used as a chronic pediatric pulmonary disease (n=46, 5-17 years, Table 1) cohort. Random participants with moderate to severe asthma were enrolled in Environmental Control as Add-on Therapy in Childhood Asthma (ECATCh) (NCT02251379), a randomized clinical trial comparing controller medication alone to controller medication plus home-based environmental intervention. All subjects had moderate to severe persistent asthma, and were on stepwise

chronic medical therapy (mean step 5 corresponding to inhaled corticosteroid and long acting beta agonist).(14) Samples were collected with Johns Hopkins IRB approval with informed consent and with subject assent where appropriate.

Methods:

Lab methods:

Serum samples were assayed for total IGF1, and IGFBP2 using commercial ELISAs (R & D Systems, Minneapolis, MN. Cat # DGB200, Human IGFBP-2; Cat # DG100, Human IGF1). Samples were assayed according to manufacturer instructions, in the same lab and by the same technician. Assays were analyzed using KC4 (Bio-Tek Instruments, Winooski, VT). All assays were blinded to clinical outcomes and only unblinded for statistical analysis.

Statistical Analysis:

Total IGF1 and IGFBP2 concentrations are presented as median and interquartile range (IQR). There are normative values for IGF1 based on age and sex; thus, IGF1 values were converted to Z-scores to compare the PAH Biobank to a healthy population, and to compare the asthmatic subject to the PAH Biobank. Normative values are not available for IGFBP2. Biomarkers were natural log transformed for regression analysis in order to normalize distribution. Demographic and functional data are presented as median and IQR, or median, percent, and range as appropriate. The results from receiver operating characteristic (ROC) curves and Youden analysis were used to determine the sensitivity and specificity of IGF1 and IGFBP2 at discriminating PAH from controls. Differences in categorical variables were assessed using Wilcoxon signed-rank test, or for continuous variables, Spearman’s rank correlation. In the PAH Biobank a subset of subjects (n=29) had hemodynamic data collected within 6 months of the enrollment blood sample; this subset was used to analyze hemodynamic data against biomarker levels. Regression analysis was performed, adjusted for age and sex, using linear and logistic regression for continuous and categorical variables respectively. Kaplan-Meier analysis was performed to evaluate time to a composite clinical worsening outcome of death, transplant, or palliative shunt (Pott’s shunt or atrial septostomy) with biomarkers dichotomized at the median and follow-up time of 60 months. Cox proportional hazard model, adjusted for age, sex, and PAH subtype was used to assess risk of the composite clinical worsening outcome based on biomarker level. A P-value less than 0.05 was considered statistically significant. Statistical analysis was performed using Stata (Version 15.1; 2018; StataCorp, LLC, College Station, TX).

Results:

Subject Demographics:

All PAH Biobank subjects were diagnosed with WSPH group 1 PAH, with serum samples collected at enrollment. There were 175 PAH Biobank and 46 asthmatic controls available for analysis. The demographic characteristics of the PAH Biobank cohort and the control cohort are shown in Table 1. Anthropometric data is presented for those with clinical evaluation within 6 months of enrollment (n=29) to correspond with hemodynamic and functional data. The PAH Biobank subjects had a median age of 12 years, with 59% female

subjects. The median age and gender were not significantly different between the PAH Biobank and the controls. The PAH Biobank subjects were comprised of 48% idiopathic PAH (IPAH), and 46% with associated PAH (APAH). The APAH group was predominantly congenital heart disease (40%), with no shunt (14%), unrepaired shunt (6%) and repaired shunt (20%).

Hemodynamic and functional measures are shown in Table 1. The PAH Biobank subjects with cardiac hemodynamics within 6 months of enrollment (n=29) had a mean pulmonary artery (mPAP) of 45mmHg, and a median pulmonary vascular resistance index (PVRi) of 10.7 WU*m^2, consistent with severe pulmonary hypertension. Functionally, within 6 months of enrollment, a six-minute walk distance (6MWD) was available for 80 subjects over 8 years of age with a mean of 422 meters. 34% of the PAH Biobank cohort were treated with a prostacyclin analogue (PCA) as another measure of disease severity.

Serum IGF proteins in Pediatric PAH:

The lower limits of detection for the IGF1, and IGFBP2 assays were 18.2ng/mL, and 4.4 ng/ml, respectively, with inter-plate coefficients of variation of 5 % for IGF1, and 3.6% for IGFBP2. IGF1 values in the control cohort were consistent with the normal clinical IGF1 concentration range (IQR 100-600ng/mL) (15) and was confirmed by calculating Z-scores for the IGF1 value for each subject (Supplementary Figure 1). The results of the serum IGF1, and IGFBP2 concentrations are detailed in Figure 1A–B and Table 1 for both the PAH Biobank and asthmatic control (median and IQR) cohorts (Figure 1A–1B). IGF1 was significantly lower in the PAH Biobank compared with pulmonary disease controls (Table 1, Figure 1A, $p<0.0001$). The Z-score distribution of IGF1 was lower in the PAH Biobank compared to a normal distribution and was significantly lower in the PAH Biobank compared to the asthmatic subjects ($P<0.001$, Supplementary Figure 1). IGFBP2 was significantly elevated in the PAH Biobank cohort compared with the pulmonary disease controls (Table 1, Figure 1B, $p<0.0001$). Within PAH subtypes, IPAH subjects had a slightly higher IGF1 (131ng/mL, 87-183, $p=0.014$). There were no significant differences in IGF1 or IGFBP2 between other disease subtypes, although the cohorts were too small to draw conclusions. There was no significant difference in IGF1 and IGFBP2 between congenital heart disease subjects with a shunt, a repaired shunt, or no shunt (Supplementary table 1).

IGFBP2 discriminates PAH from pulmonary disease controls:

To determine if IGF1, and IGFBP2 could discriminate PAH from controls, we used IGF1, and IGFBP2 values in the PAH Biobank and the control cohort to generate a ROC curves (Figure 2A–2B). Serum IGF1 was able to identify the presence of PAH versus controls with an AUC of 0.82 ($P<0.001$), with the optimal cutoff established by Youden analysis of 177.3 ng/mL. This IGF1 threshold had a sensitivity of 73.9% and a specificity of 78.3%. IGFBP2 was able to identify the presence of PAH from the controls with an AUC of 0.80 ($P<0.001$), with the optimal cutoff established by Youden analysis of 185 ng/mL. This IGFBP2 threshold had a sensitivity of 72.2% and specificity of 80.4%.

IGF1 and IGFBP2 correlate with hemodynamic severity:

We evaluated the correlation of IGF1, and IGFBP2 with major hemodynamic variables in the PAH Biobank. Spearman Rho values are shown in Table 2 for PAH Biobank subjects with cardiac catheterization within 6 months (n=29, Table 2) of enrollment. IGF1 was positively correlated with cardiac output and negatively correlated with mPAP, PVR and PVRi, whereas IGFBP2 was strongly negatively correlated with cardiac output and positively correlated with PVR and PVRi.

When linear regressions were adjusted for age and sex (Table 2, n=29), IGF1 was negatively associated with mRAP, mPAP, PCWP, and PVR. IGFBP2 was positively associated with PVR and negatively associated with cardiac output, but had no significant association with either mPAP, mRAP, or PCWP.

IGFBP2 concentration correlates with prostacyclin use:

Continuous infusion/inhalation prostacyclin therapy (IV/SQ PCA) was used as a marker of more severe disease. We explored the relationship of serum IGFBP2 levels and IV/SQ PCA therapy compared with any other combination of enteral therapies (Figure 3A). 49% of PAH subjects (n=85) were treated with PCA with 34% (n=59) receiving IV/SQ PCA. Only 1 subject was treated exclusively with a PCA. Serum median IGFBP2 concentration was significantly higher in the IV/SQ PCA administration groups compared to any other therapies without PCA (Figure 3B).

Serum IGFBP2 concentrations were associated with functional outcomes and mortality:

As shown in Table 2, using a linear regression model adjusted for age and sex, higher IGFBP2 was significantly associated with shorter 6MWD. For every 2.5-fold increase in IGFBP2 (1 natural log unit), subjects had a 140-meter shorter 6MWD (p=0.008). IGF1 was positively associated with 6MWD with subjects walking 130 meters more for every natural log increase in IGF1 (p=0.017).

IGFBP2 was positively associated with mortality with a log-odds of 1.193 (p=0.016). Thus, the odds ratio of death with 1-natural log unit higher IGFBP2 was 6.78. The median IGFBP2 concentration in subjects who died was 540ng/mL compared with 262ng/mL in survivors (p=0.009). For subjects with an IGFBP2 concentration in the top quartile (>379ng/mL), the relative risk of death was 12.4 (p=0.023, 95% CI 1.4 to 107; NNT [harm] 11.6) compared with subjects with an IGFBP2 concentration in the bottom 3 quartiles. Analysis of the composite clinical worsening outcome (death, transplant, palliative shunt) was conducted using a Cox proportional hazard model, adjusted for age, sex, and PAH subtype (Supplementary Table 2). For every 2.5-fold increase in IGFBP2, the hazard ratio for death, transplant, or shunt was 8.8, (Supplementary table 2, 95% CI 2.7- 28.6; p<0.001) indicating IGFBP2 was independently and strongly predictive of clinical worsening over time. The Cox proportional hazard model was not significant for IGF1 (hazard ratio 0.45 95% CI 0.16-1.3, p=0.13). In Kaplan-Meier analysis (Figure 4), IGFBP2 above the median was associated with time to death, transplant, or palliative shunt (p=0.02).

Discussion:

Pulmonary arterial hypertension is a severe disease with an extremely high burden of morbidity and mortality. We sought to find new circulating biomarkers which may lead to a better understanding of PAH pathobiology. IGF's are an interesting target because of their essential roles in myocardial function and metabolism, and smooth muscle and endothelial cell growth(8). This study demonstrates, for the first time, dysregulation of IGF1 and IGFBP2 in pediatric PAH and that IGFBP2 is elevated in pediatric PAH, with a significant association with disease severity and death.

IGF1 is a ubiquitous protein expressed as part of the pituitary growth hormone axis (6, 16) where growth hormone stimulates release of IGF1 from the liver.(6) IGF1 is mediated by binding the cell surface IGFR1 tyrosine kinase coupled receptor, triggering a signaling cascade influencing growth.(6) IGF1 exerts particular effects in the heart; IGF1 is necessary for fetal/neonatal cardiomyocyte proliferation and maturation, upregulation of IGF1 is seen in animal models of ventricular hypertrophy, and increased pressure or volume overload, while IGF1 deficiency results in heart failure and death (6, 17, 18). Equally important, IGF1 modulates vascular tone and nitric oxide production, which when abnormal, contribute to pulmonary hypertension.(16) In this study, lower IGF1 was associated with higher pulmonary artery pressures, and lower cardiac output, reflecting the previously described essential role for IGF1 in the cardiovascular system (8).

While IGF1 is essential for cardiac function and growth, IGFBPs have been shown to have an evolving IGF1 independent role in adverse growth and cellular remodeling of the heart, and worse outcomes after cardiovascular events.(19) In normal physiology, IGFBP2 has been shown to be mostly growth inhibitory, binding IGF1 which is mitogenic for vascular growth. In disease, however, IGFBP2 expression, and regulation may be very altered, with increased IGF1 independent effects on vascular growth and function.(20) For example, IGFBP2 plays a role in dysregulated vascular growth in aggressive cancers. In human neuroblastoma cells, IGFBP2 overexpression upregulated VEGF transcription while in vitro studies have shown increased levels of IGFBP2 activating integrin receptors and promoting cell migration required for new vessel growth.(20–22) Other studies show regulation of IGFBP2 in response to HIF-1α, causing upregulation of IGFBP2(9), while an in-silico model suggested that upregulation of IGFBP2 potentiated HIF-1α expressions, further driving vascular growth.(23)

In this study we demonstrate that IGF1 is decreased and IGFBP2 is elevated in pediatric PAH subjects compared with subjects with another chronic pulmonary disease, and that IGFBP2 is significantly associated with markers of disease severity (6MWD, PVR, CO, use of continuous infusion therapy, mortality). The etiology of PAH mediated IGFBP2 elevation is currently unknown. But the pattern seen in these subjects is clear; in PAH there is an elevated IGFBP2 concentration, and a lower available IGF concentration. IGFBP2's hemodynamic and functional associations may also be independent of IGF concentration, a pattern of vascular dysfunction seen in patients with pulmonary fibrosis as well as multiple cancers(9)(11). Although IGFBP2 has been shown to drive abnormal vascular growth in cancer, it has not been evaluated in pulmonary hypertension. However the same mechanisms

of endothelial and vascular smooth muscle dysfunction may be relevant, namely abnormal pulmonary vascular development, metabolic dysregulation, abnormal vascular tone, and in PAH, resultant right ventricular failure.(6, 13, 16) Thus, there may be a two-fold insult from IGFBP2 mediated dysregulated vascular function, and a relative deficiency of IGF from increased IGFBP2 binding, causing hemodynamic compromise with increased pulmonary vascular resistance and decreased cardiac output. The reciprocal associations of functional outcomes of IGF1 and IGFBP2 suggest that dysregulation of this axis, with increased IGFBP2, and resultant worsening IGF availability in PAH may be a mechanism of worsening disease. Taken together the IGF axis is essential for normal cardiopulmonary development and function and is dysregulated in pediatric PAH with strong associations with severity and survival.

This study is the first to explore IGF1 and IGFBP2 in pediatric pulmonary arterial hypertension. While the overall size of the cohort is large for a pediatric PAH study, limitations include the diverse nature of pediatric PAH, as well as the limited number of subjects with contemporaneous hemodynamic and functional data. Further, this has not yet been validated in an independent cohort. The study is cross sectional, with subjects enrolled at different phases of disease, and blood samples collected only at enrollment. Thus, it is not possible to associate IGFBP2 longitudinal changes with response to treatment. There were 6 deaths in the PAH Biobank group limiting the power to assess mortality, although the study was well powered for the composite outcome. There was still a significant association with mortality in the PAH Biobank, which mirrors the pattern seen in adult studies. Future studies should focus on a longitudinal analysis of IGF1 and IGFBP2 in pulmonary hypertension to validate this model, assess response to treatment and to develop a better prognostic model for outcomes.

Given the essential function of IGF1 and IGFBP2 in vascular and cardiomyocyte growth, maturation and function, these proteins may play a key role in the pathogenesis of PAH. IGFBP2 may be a useful prognostic biomarker of pediatric PAH and have the potential as the focus for development of new therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

All samples and clinical data have been collected with informed consent and assent where appropriate. All cohorts have been approved by the Institutional Review Board at their respective institution and by the Johns Hopkins University Institutional Review Board.

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Impact:

- Pediatric pulmonary hypertension is a severe disease, with poorly understood pathobiology.
- There are few studies looking at the pathobiology of pulmonary hypertension only in children.
- The IGF axis is dysregulated in pediatric pulmonary arterial hypertension
- IGF axis dysregulation, with increased IGFBP2, is associated with worse clinical outcomes in pediatric pulmonary artery hypertension
- IGF axis dysregulation gives new insight into the disease process, and may be a mechanistic or therapeutic target

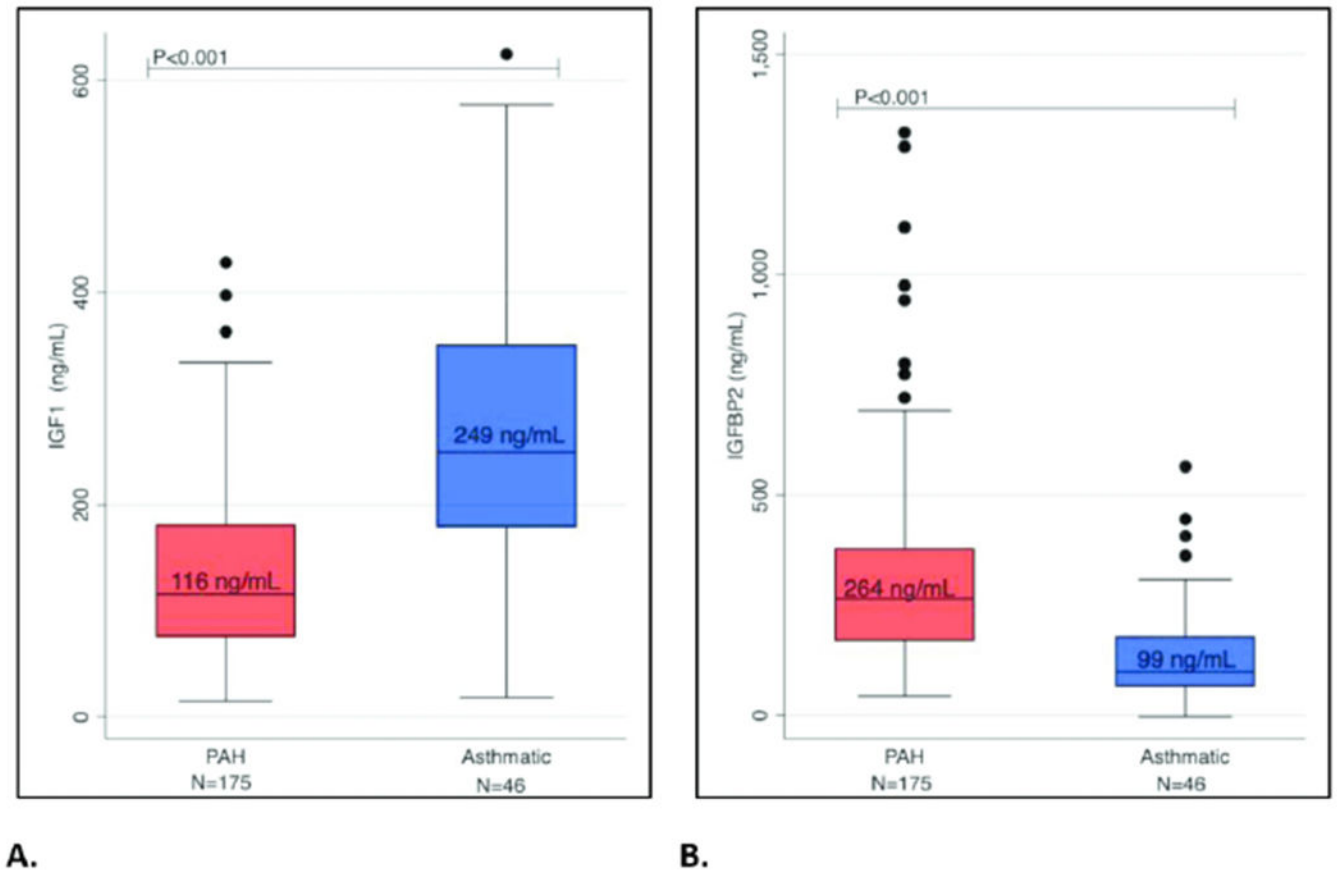

Figure 1.

IGF1 and IGFBP2 concentration (ng/mL) in PAH Biobank versus controls. (A) IGF1 concentration (ng/mL) in PAH Biobank versus asthmatic subjects. (B) IGFBP2 concentration (ng/mL) in PAH Biobank versus asthmatic subjects.

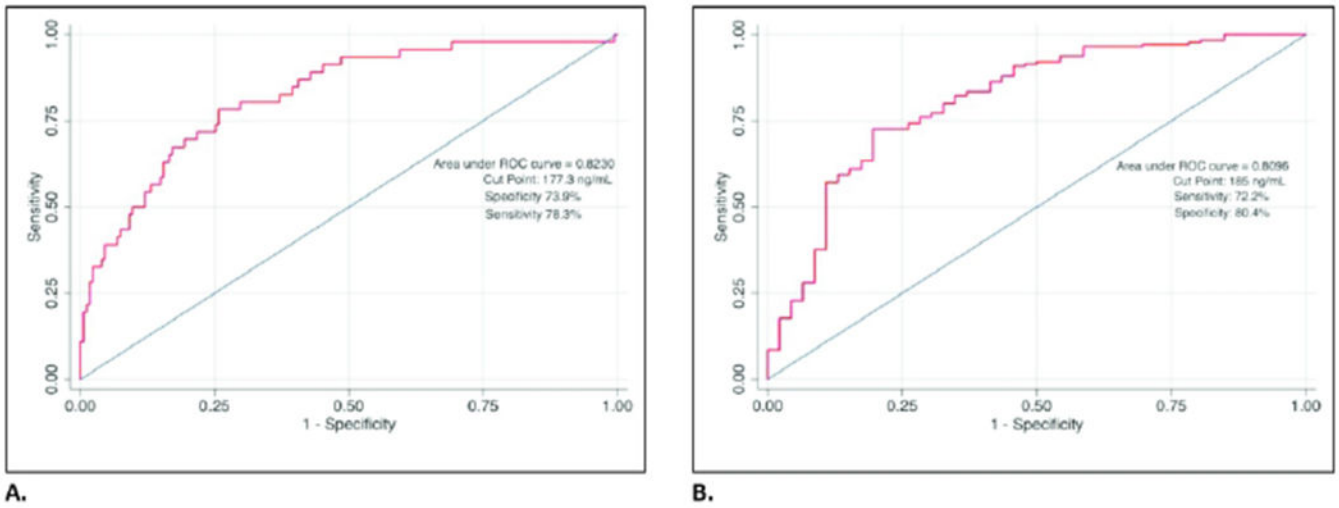

Figure 2.

ROC curve of IGF1 and IGFBP2 in PAH Biobank versus controls.

(A) ROC curve of IGF1 in PAH Biobank subjects vs. controls. AUC 0.82. Cut point for IGF1 of 177ng/mL gives sensitivity of 73.9% and specificity of 78.3%. (B) ROC curve of IGFBP2 in PAH Biobank subjects vs. controls. AUC 0.80. Cutpoint for IGFBP2 of 185ng/mL gives sensitivity of 72.2% and specificity of 80.4%.

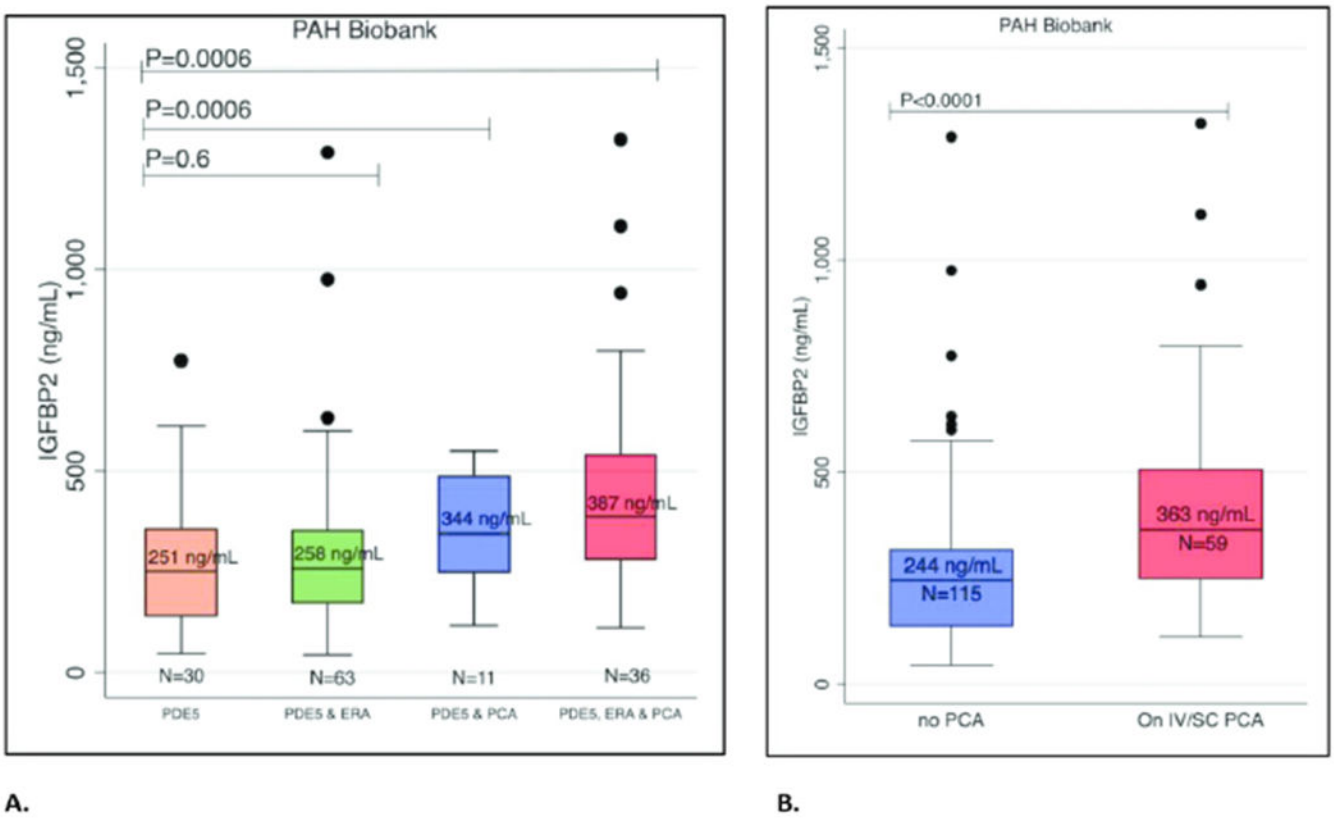

Figure 3.

IGFBP2 concentrations in PAH subjects by medication combination. (A) IGFBP2 concentration in PAH subjects on a PDE5 inhibitor, a PDE5 inhibitor and an ERA, a PDE5 inhibitor and IV/SQ PCA, or a combination of PDE5 inhibitor, ERA, and IV/SQ PCA. (B) IGFBP2 concentrations in PAH subjects on an IV/SQ PCA and any other therapy versus subject not on IV/SQ PCA and any other therapy.

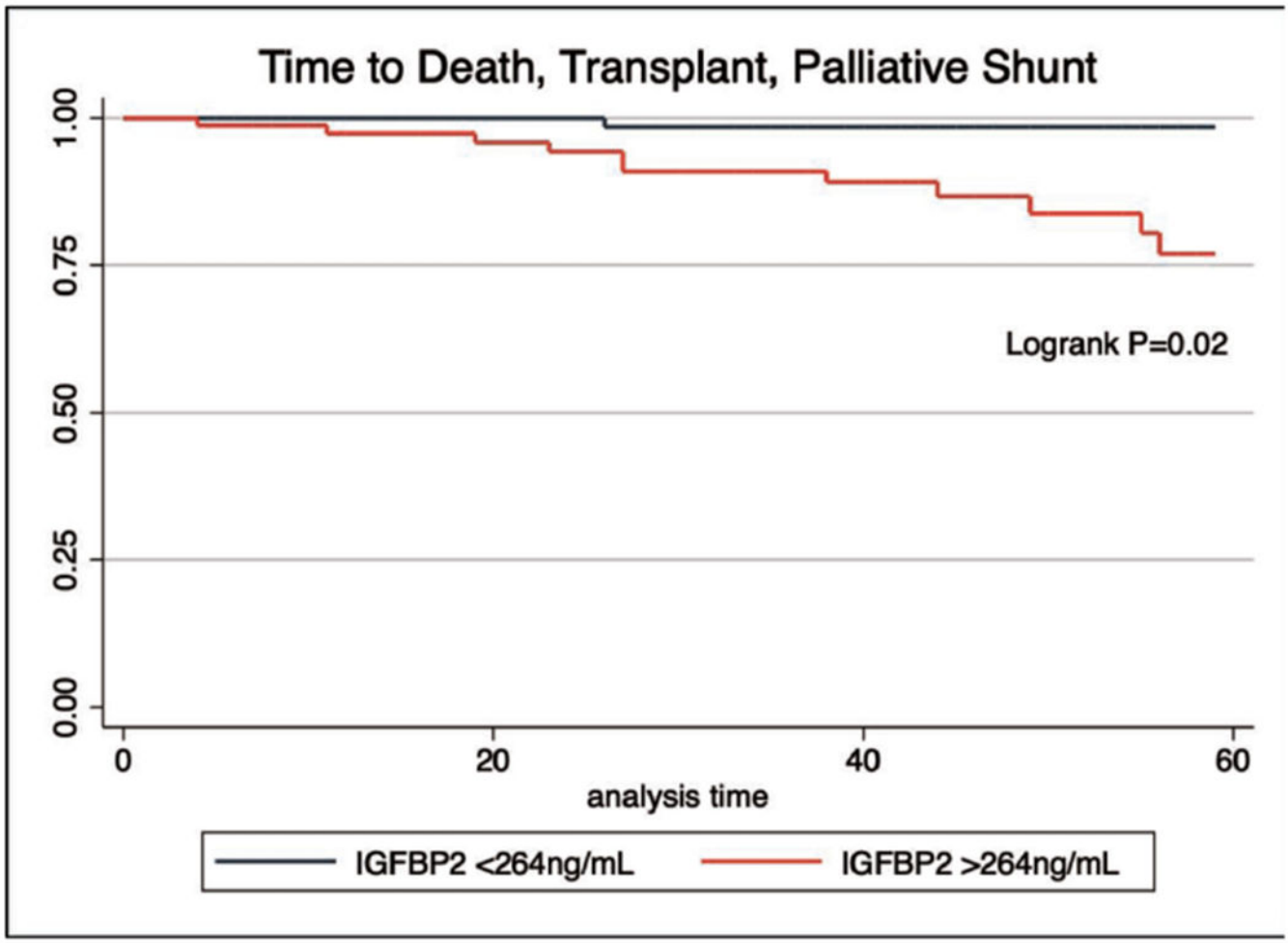

Figure 4.

Kaplan-Meier curve showing time to death, transplant, or palliative shunt (Pott's shunt or atrial septostomy) dichotomized by the median IGFBP2 level.

Table 1.

Demographics and clinical characteristics of PAH subjects and controls at enrollment

	PAH Biobank	Asthmatic Controls
Demographics		
Subjects, n	175	46
*Age, years	12 (8-15)	10 (5-17)
Sex, n female (%)	104 (59)	18 (39)
*Weight, kg	31.5 (15-51.5)	
*Height, cm	132 (102-156)	
*BSA, m^2	1 (0.6-1.4)	
NYHA FC, n I/II/III/IV (%III/IV)	21/53/52/12 (46)	
*6MWD, m (n)	442 (80)	
Deaths, n (%)	6 (3.5)	
Transplant or Shunt, n (%)	6 (3.5)	
Etiology, n (%)	-	
APAH	80 (46)	
IPAH	84 (48)	
HPAH	11 (6)	
Biomarker values		
IGF1, ng/mL	116 (75-181)	249 (178-352)
IGFBP2, ng/mL	264 (168-379)	99 (62-181)
Hemodynamics (n=29) *		
RAP, mmHg	7 (5-9)	
mPAP, mmHg	45 (35-60)	
PCWP, mmHg	8.5 (7-9)	
PVR, Wood units	12 (8-14)	
PVRi, Wood units*m^2	10.7 (5.4-16)	
Cardiac output, L/min	3.5 (2.3-4.7)	
Cardiac index, L/min/m^2	3.3 (2.7-3.8)	
Therapies, n (%)		
PDE5 inhibitor	161 (92)	
ERA	114 (65)	
IV/SC prostacyclin	59 (34)	
CCB	29 (17)	

All data presented as median (IQR) unless otherwise specified.

* Hemodynamic measurements, age, weight, height, and bsa limited to subjects with biomarkers obtained within 6-months of clinical tests, n=29

Definition of abbreviations:; NYHA FC: New York Heart Association Functional Class; 6MWD: six minute walk distance; APAH: associated PAH; IPAH: Idiopathic PAH; FPAH/HPAH: familia/heritablel PAH;; IGF1: Insulin like growth factor 1; IGFBP2: Insulin like growth factor binding protein 2; RAP: right atrial pressure; mPAP: mean pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; TR Peak gradient: Tricuspid regurgitation peak gradient; PDE5: phosphodiesterase-5; ERA: endothelin receptor antagonist; IV: intravenous; SC: subcutaneous; CCB: calcium channel blocker.

Table 2.

Correlations between biomarkers and continuous clinical variables and age- and sex-adjusted linear regressions of biomarkers and continuous clinical variables

	Unadjusted Spearman Correlation		Adjusted Linear Regression	
	IGF1	**IGFBP2**	**(ln) IGF1**	**(ln) IGFBP2**
Demographics				
Age, years	0.27, 0.15	−0.21, 0.28	4.2 (3.1-5.2, <0.001)	−1.4 (−2.6- −0.15, 0.03)
BSA, m^2	0.43, 0.02	−0.48, 0.009	0.12 (−0.09-0.3, 0.3)	−0.21 (−0.35- −0.07, 0.006)
6MWD, m *	0.5, 0.05	−0.71, 0.003	130 (28.4- 232, 0.017)	−140.4 (−235- −45, 0.008)
Hemodynamics *				
RAP, mmHg	−0.31, 0.09	0.25, 0.2	−1.7 (−3.15- −0.2, 0.03)	0.88 (−0.9- 2.67, 0.3)
mPAP, mmHg	−0.4, 0.03	0.34, 0.07	−10.5 (−19.4- −1.6, 0.02)	8.6 (−1.78- 19, 0.1)
PCWP, mmHg	−0.24, 0.2	0.01, 0.9	−1.49 (−2.7- −0.27, 0.019)	0.56 (−0.94- 2.1, 0.5)
PVR, Wood units	−0.55, 0.003	0.61, 0.001	−4.76 (−8.8- −0.7, 0.02)	4.89 (0.32- 9.4, 0.03)
PVRi, Wood units*m^2	−0.53, 0.005	0.67, <0.001	−0.79 (−7.1-5.5, 0.8)	5.0 (−1.26-11, 0.1)
Cardiac output, L/min	0.37, 0.04	−0.48, 0.008	0.37 (−0.35- 1, 0.3)	−0.73 (−1.5- 0.032, 0.03)

All data presented as Spearman correlation coefficient, p value, or regression coefficient (95% CI, p value).

* PAH Biobank limited to subjects with biomarkers obtained within 6-months of clinical tests, n=29

See Table 1 for all other abbreviations