THE ROLE OF PULMONARY ARTERIAL STIFFNESS AND SHEAR HEMODYNAMICS
IN CHILDREN WITH PULMONARY HYPERTENSION

by

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The Role of Pulmonary Arterial Stiffness and Shear Hemodynamics in Children with Pulmonary Hypertension.

Thesis directed by Associate Professor Kendall S. Hunter

ABSTRACT

Pediatric pulmonary arterial hypertension (PAH) comprises a spectrum of cardiovascular diseases associated with significant morbidity and mortality. Relatively recently has been recognized the concept of pulmonary arterial stiffness as the significant component of PAH pathogenesis. Furthermore, the stiffening process has been also associated with large – proximal pulmonary arteries. Unfortunately, the only available therapies predominantly target pulmonary vasoconstriction and lack specificity against anti-proliferative vasculopathy and vascular remodeling. Lastly, the characterization of pulmonary vascular stiffness / resistance is being typically conducted by means of invasive catheterization which carries a significant amount of risk in infants and children with severely progressed PAH.

The overarching goal of this work was to non-invasively characterize the proximal pulmonary arterial stiffness along with shear hemodynamics using phase-contrast MRI in a larger group of pediatric PAH patients and link those to right ventricular dysfunction – the major morbidity and clinically predictive markers of functional worsening.

Primary results of this work indicate that 1) proximal pulmonary arteries in children with PAH show clear evidence of elevated stiffness which is prognostic of clinical and functional worsening, 2) severity of the pulmonary arterial stiffness is associated with reduced hemodynamic wall shear stress, and 3) the longitudinal changes in wall shear stress and stiffness through-out the disease progression are interrelated. Non-invasive flow hemodynamic evaluation
via PC-MRI is feasible in children with PAH and might become a routine component of clinical evaluation in pediatric PAH, providing comprehensive evaluation of pulmonary arterial stiffness, flow hemodynamic status, and clinically meaningful biomarkers predictive of functional worsening. Most importantly, observed relationship between flow mediated shear stress and vascular stiffness suggests that novel therapeutic strategies targeting distal vascular remodeling might be further beneficial for the restoration of proximal pulmonary arterial compliance as well.

The form and content of this abstract are approved. I recommend its publication.

Approved: Kendall S. Hunter
DEDICATION

This work is dedicated to Annalise McKinney and her family. Annalise and many other children who fought and are fighting this terrible disease served as the primary motivation for this work.
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CHAPTER I
INTRODUCTION

Pediatric Pulmonary Arterial Hypertension

Pediatric Pulmonary Arterial Hypertension (PAH) is a relatively rare and progressive disease with worse prognostic survival rates when compared to the adult population\textsuperscript{1,2}. PAH is typically associated in children with diverse congenital cardiac, pulmonary, and systemic diseases. Due to heterogeneity and complexity of congenital heart lesions, a comprehensive classification system considering the pediatric pulmonary vascular disease was developed at the Panama Pulmonary Vascular Research Institute\textsuperscript{3}. Two most prevalent subtypes of pulmonary hypertension include idiopathic PAH (IPAH) and PAH associated with congenital heart disease with respective prevalence of 0.7 and 2.2 cases per million\textsuperscript{4}. The most recent guidelines published by American Heart Association and American Thoracic Society define pediatric PAH by a mean pulmonary arterial pressure (mPAP) $\geq$ 25 mm Hg, pulmonary arterial wedge pressure < 15 mm Hg, and pulmonary vascular resistance index (PVRi) > 3 units$\times$m\textsuperscript{2}. Given the lower systemic blood pressure in children, some prefer to further apply PVR to systemic vascular resistance ratio > 0.5\textsuperscript{5}. Survival is dependent on disease severity, diagnoses, and treatment strategy with a 5-year freedom from lung transplantation survival rate of 81% and overall 5-year survival rate varying between 62 to 90%\textsuperscript{6}.

PAH is primarily characterized by the progressive remodeling of small resistant pulmonary arteries featured by chronic vasoconstriction and obliteration of arterial lumen by means of endothelial and medial thickening\textsuperscript{1,8}. While the pathogenesis of the PAH related vascular remodeling is a complex phenomenon, the resulting effect is typically described by endothelial dysfunction, smooth muscle and fibroblast proliferation, endothelial cell apoptosis,
and significant changes in extra-cellular matrix composition\textsuperscript{7–9}. \textbf{Relatively recently has been recognized the concept of pulmonary arterial stiffness as the significant component of PAH pathogenesis\textsuperscript{10,11}. Furthermore, the stiffening process has been also described to effect large – proximal pulmonary arteries\textsuperscript{12}. Widespread vascular remodeling leads to elevated PVRi and reduced pulmonary arterial compliance resulting in significantly elevated afterload on the right ventricle (RV), which after relatively short-lived compensatory stage progresses to a decompensation stage with reduced cardiac output and eventually heart failure and death\textsuperscript{13,14}.

The elastin dominant pulmonary arteries are more compliant than their systemic counter-parts and help to buffer the initial rise in pulmonary blood pressure (Figure 1). This typically results in delayed onset of clinical symptoms typically manifested by shortness of breath, syncope, and exercise intolerance. With the progression of the vascular remodeling, the composition of the extra-cellular matrix shifts from the elastin dominant (elasticity providing) to collagen dominant (load bearing) character resulting in stiff pulmonary arteries which escalates the stress on RV.

\textbf{Figure 1.} \textbf{A}) graph representing the non-linear elastic modules of the vasculature. Within the physiologic range, elastin fibers (black) are nearly completely stretched but only a small portion of stiffer collagen (white) is undergoing shape change. These changes outside of physiologic stress regions and at higher strain collagen engagement and stretch contribute to stiffening. The two tangent lines represent the relative stiffness of elastin (black) and collagen (white). \textbf{B}) Graph showing the stress-versus-strain behavior of fresh PAH-associated main pulmonary arterial tissue compared with non-PAH control (CTRL) pulmonary arterial tissue. Trans is the strain of transition from the elastin-dominant region (A) to the collagen-dominant region (B), associated with increased collagen engagement within the physiologic strain region. \textit{Schäfer et al. Curr Hypertens Rep (2016) 18:4}. 
The ability of the RV to adapt to increased pulmonary arterial load has been recently identified as the most important determinant of clinical outcomes\textsuperscript{15}. Furthermore, the ventricular-vascular coupling, defined as the ratio between pulmonary arterial elastance and RV contractile function, has been shown as a strong prognostic biomarker of clinical events\textsuperscript{16,17}. In general, RV load-dependent and pulmonary arterial stiffness biomarkers have been recognized as the most clinically useful independent prognostic markers in adults with PAH\textsuperscript{18}. However, the role of proximal pulmonary arterial stiffness in pediatric population is yet to be determined. Pioneering comprehensive studies using combined catheterization and echocardiography suggest that both resistive and stiffness components of the RV afterload play important role in pediatric PAH\textsuperscript{19,20}.

Aside of oxygen and nitrous oxide therapy, three classes of drugs are being typically applied in the clinical setting and include 1) phosphodiesterase-5 inhibitors which promote nitrous oxide signaling and vasodilation, 2) endothelin receptor antagonists which interfere with endothelin-1 signaling promoting vasoconstriction, and 3) prostanoids which stimulate vasodilation via cyclooxygenase pathway. Unfortunately, the only available therapies predominantly target pulmonary vasoconstriction and lack specificity against anti-proliferative vasculopathy and vascular remodeling\textsuperscript{7,13,21}.

The initial screening algorithm involves echocardiogram, ECG, and chest X-ray typically followed by pulmonary function test\textsuperscript{1,22}. However, positive findings need to be confirmed by cardiac catheterization and acute vasoreactivity testing which are typically associated with laboratory testing for brain natriuretic peptide and N-terminal pro-brain natriuretic peptide levels\textsuperscript{23}. Routine clinical follow-up typical involves echocardiography, 6-minute walk test, and further laboratory studies. Based on patient population, further imaging techniques such as CT or
MRI can be considered to address the global lung parenchyma and ventricular function respectively.

**Challenges in Pediatric Pulmonary Arterial Hypertension**

Nearly every aspect of pediatric PAH is amalgamated by additional challenges when compared to adult PAH counter-part. Primarily, all therapeutic methods applied in pediatrics are derived from adult PAH studies and their application / translation to the routine clinical use is complicated by longer and extensive trials\(^2,5,13\). Furthermore, the study end-points sensitive to hard, moderate, or soft clinical outcomes in pediatric PAH patients are yet to be determined. Lower incidence rate in the pediatric population further complicates prospective longitudinal studies and identification of independent prognostic biomarkers.

The complexity associated with congenital heart lesions further complicates PAH evaluation particularly when associated with the timing and severity of pulmonary vascular injury through-out childhood development. **Cardiac catheterization required for ultimate diagnoses and assessment of PAH severity carries additional risks in pediatric population associated with anesthesia burden, invasiveness mediated by catheter interventions, and should be performed in specialized institutions with PAH focused centers**\(^1,22,23\). Furthermore, increasing amount of evidence suggests that flow based hemodynamic indices derived by catheterization by Fick and thermodilution may be less accurate with increasing disease severity (Figure 2 – next page)\(^24,25\). **Consequently, novel monitoring and diagnostic non-invasive modalities with high diagnostic sensitivity and specificity providing clinically meaningful and prognostic biomarkers are urgently needed in this patient population.**
The Role of Flow Mediated Wall Shear Stress

The interplay between flow mediated forces and vascular remodeling has been of major interest since the beginning of the 21st century when Malek et al published the first comprehensive review discussing the relationship between the hemodynamic shear stress, vessel wall biology, and pathogenesis of atherosclerosis26. **Flow mediated wall shear stress (WSS) is indeed one of the major modulators of vessel wall biology which can promote vascular remodeling toward stiffer character.** Shear stress is defined as the force per unit area induced when a tangential force mediated by blood flow acts on a surface represented in this scenario by vascular endothelium. Conventional biomechanical definition of WSS is the product of shear rate...
at the endothelial surface and blood viscosity. Shear drag forces stimulate by means of mechanotransduction endothelial receptors (PECAM-1), responsible for triggering the endothelial signaling cascades (Figure 3)\textsuperscript{27}.

![Diagram](image)

**Figure 3.** Shear mediated mechanotransduction or “flow signaling” is a very potent stimulator of endothelial signaling cascades capable of vascular wall remodeling. *Davies et al. Nat Clin Pract Cardiovasc Med. 2009;6(1):16-26.*

Hemodynamic WSS and endothelial surface function in homeostatic equilibrium, i.e. neither high or low WSS is well tolerated by endothelium. Specifically, endothelial mechanotransduction appears to be sensitive to magnitude, directional, and temporal shear stress deviations\textsuperscript{28,29}. Indeed, mechanotransduction driven endothelial and extra-cellular matrix remodeling in vascular diseases has been described in several *in-vitro* and animal studies\textsuperscript{27,30,31}. Importantly, in both systemic and pulmonary circulations, shear mediated response has a spatially heterogeneous response, i.e. endothelial cells in large compliant artery will have different responsiveness to shear abnormalities than those at the level of resistant arteries and arterioles\textsuperscript{32-34}.
Currently, the flow mediated remodeling of pulmonary and systemic vasculature is already recognized phenomenon applicable to broad range of vasculopathies. On the systemic side, WSS has been linked with the progression and pathobiology of atherosclerosis, bicuspid aortic valve aortopathy, aortic valve stenosis, aneurysmal formation, abnormal aortic flow patterns in chronic obstructive pulmonary disease, and even in cerebral vasculopathies\textsuperscript{30,35–39}. More recently, WSS has been investigated in the context of pulmonary hypertension with regard to pulmonary arterial remodeling\textsuperscript{32,40}. \textbf{In the context of pulmonary hypertension} microvascular endothelial cells are exposed to abnormally high WSS whereas the endothelial cells lining large proximal pulmonary arteries are exposed to reduced WSS\textsuperscript{32,41,42}. It has been hypothesized that such a changes in flow hemodynamic environment might be responsible for augmentation (initiation) of vascular remodeling and stiffness development in pulmonary vasculature. This phenomenon should be of particular interest given the increasing amount of evidence from dynamic imaging studies describing the flow through proximal pulmonary arteries as highly turbulent, chaotic, and recirculative giving a rise to abnormally low and oscillatory shear stress.

\textbf{Figure 4.} Differences in flow hemodynamic pattern between normal healthy subject A) and age-matched patient with idiopathic PAH B). Flow through the right ventricular outflow tract-pulmonary artery axis is chaotic and recirculative, which further contributes to reduced and oscillatory wall shear stress.
Measuring Flow Mediated Shear Stress and Stiffness by Magnetic Resonance Imaging

Phase-contrast magnetic resonance imaging (PC-MRI) – velocity encoded imaging is arguably considered the gold standard method for flow evaluation due to its high sensitivity to non-laminar flow environment and excellent reproducibility in phantom in-vitro studies\textsuperscript{43,44}. Furthermore, PC-MRI does not require intravenous contrast agents for signal enhancement and represents non-invasive method for accurate evaluation of flow hemodynamic function even at the presence of complex cardiac lesions. PC-MRI is most frequently applied at the clinical setting for evaluation of pulmonary-to-systemic shunts, assessment of regurgitation fraction, and calculation of stroke volume (cardiac output). The generation of flow hemodynamic waveforms stems from measuring of the through plane velocity encountered in selected vessel lumen. The luminal contours are typically segmented from corresponding magnitude images throughout the entire cardiac cycle (Figure 5). Since every pixel within the segmented vascular contour is representative of velocity encountered at the pixel defined location within the vessel lumen, one can calculate the flow rate through each pixel simply as a product of the through plane velocity vector $\vec{v}_\perp$ and pixel area size $a_i$:

![Figure 5](image)

**Figure 5.** A) Flow calculation from PC-MRI requires segmentation of luminal contour of vessel of interest from respective magnitude and phase images. B) Flow through each pixel is calculated as a product of perpendicular pixel velocity vector and pixel area. C) Total flow is computed as summation of all pixels within segmented vessel contour.
\[ Q_i = \vec{v}_i \times a_i \]  \hspace{1cm} (1)

Volumetric flow rate at a particular time \( t \) during cardiac cycle is then calculated as the summation of pixel-velocity products within segmented lumen:

\[ Q_{Rot,t} = \sum_{i=1}^{N} Q_i = \sum_{i=1}^{N} \vec{v}_i \times a_i \]  \hspace{1cm} (2)

Consequently, PC-MRI can provide flow and area waveforms which can be further applied to calculate shear hemodynamic indices and stiffness based parameters, respectively. Hemodynamic WSS is typically calculated from PC-MRI data-sets as a product of hemodynamic shear-rate and blood viscosity \( \eta \):

\[ WSS = \frac{du}{dy} \eta \]  \hspace{1cm} (3)

Viscosity could be estimated from patient specific hematocrit or could be uniformly applied to resemble typical viscosity value approximately equal to 0.032 g/cm/s\(^4\). Graphical representation of WSS calculation at various locations in main pulmonary artery is depicted in Figure 6.

**Figure 6.** Graphical representation of WSS calculation inside the main pulmonary artery. WSS is calculated as a product of hemodynamic shear at the vessel wall and viscosity.

Calculation of hemodynamic WSS using PC-MRI has been previously validated and applied on the systemic side in patients with bicuspid aortic valves, hereditary tissue diseases,
aortic stenosis, and aneurysmal aortopathies\textsuperscript{37,43,45,47}. With regard to PAH, 2D PC-MRI and four-dimensional flow MRI have been applied to measure WSS in proximal pulmonary arteries and found significantly reduced WSS in proximal pulmonary arteries\textsuperscript{48,49}. However, all previously conducted WSS in PAH have failed to correlate shear hemodynamic with stiffness indices. Most, importantly similar studies have not been reproduced and translated to the pediatric PAH. The detailed characterization of pulmonary vascular stiffness indices by non-invasive PC-MRI in children is yet to be determined as well.

**Specific Aims**

As described in previous sections, pediatric PAH studies suffer from lack of precise characterization of proximal pulmonary vascular stiffness and flow hemodynamic characterization of RV – pulmonary axis. This is mainly due to invasiveness of right heart catheterization and its associated sedation / anesthesia requirement and due to the fact that pulmonary arterial stiffness one of the prominent features of the diseases has been ignored despite being shown to be an important prognostic factor. Furthermore, the accuracy of flow and stiffness measurements derived from catheterization is limited mainly due to inaccuracies and assumptions associated with the flow calculations. Indeed, the role of proximal pulmonary arterial stiffness and its relationship with the flow related shear hemodynamics in pediatric PAH has not yet been described.

As a consequence, the overarching goal of this work is to characterize the proximal pulmonary arterial stiffness along with shear hemodynamics in a larger group of pediatric PAH patients and link those to RV dysfunction -- major morbidity and clinically predictive
marker of functional worsening. The following specific investigations are described in the subsequent chapters:

Chapter 2 - Aim 1 **Hypothesis: Proximal pulmonary arteries are stiffer in children with PAH when compared to healthy controls and the stiffness severity is reflective of clinical outcomes.** **Approach 1:** To retrospectively characterize proximal pulmonary vascular stiffness via PC-MRI data sets in pediatric PAH patients using wave-intensity analysis. **Approach 2:** Assess the prognostic ability of PC-MRI derived stiffness indices to detect functional worsening using univariate proportional hazard analysis.

Chapter 3 - Aim 2 **Hypothesis: Hemodynamic WSS is abnormal in pediatric PAH patients and is associated with pulmonary arterial stiffness.** **Approach:** Characterize WSS in proximal pulmonary arteries non-invasively using PC-MRI in pediatric PAH patients and correlate with pulmonary stiffness and RV functional indices.

Chapter 4 - Aim 3 **Hypothesis: Longitudinal changes in pulmonary arterial stiffness are associated with changes in WSS hemodynamics.** **Approach:** Measure WSS and stiffness in pulmonary arteries via PC-MRI at different time points and assess the relationship between the respective changes using mixed-model effect methodology.

Improved understanding of the proximal pulmonary arterial stiffness in pediatric PAH and its association with flow hemodynamic forces may improve the therapeutic planning, development of novel non-invasive clinical biomarkers, and identification of the therapeutic targets focusing on reverse pulmonary vascular remodeling. It is to be hoped that features of this work are provocative enough to stimulate a discussion, which is of highest importance in the present state of knowledge.
CHAPTER II

NON-INVASIVE WAVE INTENSITY ANALYSIS PREDICTS FUNCTIONAL WORSENING IN CHILDREN WITH PULMONARY ARTERIAL HYPERTENSION

Introduction

Pediatric pulmonary arterial hypertension (PAH) contributes to poor long-term clinical outcomes in a wide spectrum of childhood diseases\(^1,^5,^2^2\). The cardinal feature of PAH is widespread pulmonary arterial remodeling characterized by distal luminal narrowing, which leads to increased pulmonary vascular resistance (PVR)\(^8,^3^3\). Recent studies evaluating pulmonary vascular stiffness in adult patients with PAH revealed that elevated abnormal wave reflections originating from pulmonary arterial branches contribute to increased right ventricular (RV) afterload\(^5^0,^5^1\). Similar studies are lacking in children with PAH partially because wave intensity analysis (WIA), which is necessary to assess vascular stiffness, requires an invasive pressure-derived waveform along with simultaneous flow measurements. Furthermore, speed wave propagation can be accurately analyzed using wave propagation method using phase-contrast MRI, but this method requires larger vessel length and is more applicable to the central aorta and is therefore less reliable for pulmonary vessels in pediatric setting.

Originally described by Parker and Jones\(^5^2\), WIA can describe the formation of waves propagating through the arterial system by separating respective pressure and flow waveforms in time domain rather than in the frequency domain that is traditionally applied for impedance analysis\(^5^3,^5^4\). Furthermore, Quail et al showed that WIA can be performed in pulmonary arteries non-invasively by using phase-contrast MRI-derived flow and area waveforms\(^5^5\). Respiratory navigated free breathing sequences allow for optimal temporal resolution and the generation of high contrast images for pulmonary artery wall delineation, which enables the ability to perform
WIA in children with breathing difficulties or who require anesthesia. This application would be of great benefit in children with PAH due to its non-invasive nature and ability to comprehensively assess the pulmonary vascular stiffness beyond traditional invasive markers of compliance. Importantly, hemodynamic indices reflective of pulmonary vascular stiffness have been progressively recognized as one of the strongest prognostic markers of clinical outcomes in both adult and pediatric PAH populations\textsuperscript{56,57}.

Consequently, the purpose of this study was to 1) characterize pulmonary vascular stiffness using WIA in children with PAH who underwent phase-contrast MRI and right heart catheterization on the same day; 2) compare WIA indices with traditional catheterization and MRI -derived hemodynamics; and 3) assess the ability of WIA indices to predict functional worsening in children with PAH. We hypothesized that children with PAH will display abnormal wave reflection when compared to healthy control subjects, and that WIA indices will reflect traditional cardiac catheterization metrics and functional worsening. Improved understanding of pulmonary vascular stiffness in children with PAH may enhance longitudinal clinical management and aid in future studies of novel therapeutic strategies to reverse pulmonary arterial remodeling.

**Methods**

As a part of a retrospective study, children followed by the Pulmonary Hypertension Clinic at Children’s Hospital Colorado between January 2010 and December 2017, underwent clinically indicated cardiac MRI followed by right heart catheterization as dictated by their clinical status. The primary diagnosis of PAH was established after evaluation which included echocardiography and a prior cardiac catheterization per accepted guidelines\textsuperscript{22}. Children with
PAH associated with congenital heart disease who required surgical or cardiac catheterization interventions involving the pulmonary arteries were excluded from the analysis. MRI control subjects were prospectively recruited via a medical campus advertisement and were included if they did not have any known underlying cardiac, pulmonary, or systemic disease. This study was approved by the Colorado Multi-Institutional Review Board, and all subjects provided written informed consent.

**MRI Protocol**

MRI acquisitions were performed using a 1.5 or 3.0 Tesla magnet system (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany; Ingenia, Philips Medical Systems, Best, The Netherlands). The flow hemodynamic evaluation acquisition protocol was performed by applying gradient echo ECG–gated sequence with diaphragmatic respiratory navigation to obtain respective magnitude and phase velocity encoding maps. The acquisition plane for the WIA was positioned in the mid main pulmonary artery (MPA) to secure sufficient distance from the pulmonary valve. A typical sequence for free-breathing PC-MRI with Cartesian encoding and retrospective sorting had a temporal resolution of 14 to 20 ms with 40 to 50 phases, echo times of 2.2 to 3.5 ms, matrix 160×256, flip angle of 25°, and 100% of the k-space sampling. Depending on patient size and the field of view (128–225×210–360 mm), the cross-sectional pixel resolution was found to be 0.82×0.82 to 1.56×1.56 mm² with a slice thickness of 5 mm. Final time of acquisition varied on heart rate and gating efficiency and ranged from 2 to 3 minutes. Velocity-encoding values were adjusted to avoid aliasing artifact (typical values ranged from 100 to 150 cm/s). The ventricular volumetric and functional analysis was performed using
steady-state free precession images with standard short-axis stacks with coverage of the ventricles from base to apex.

Wave Intensity Analysis

The overall schematic of WIA processing is depicted in Figure 7.

**Figure 7.** Wave Intensity Analysis workflow. A) Segmented magnitude and phase images from acquired phase-contrast MRI were required to create flow and area waveforms B) which were further interpolated to achieve 5 ms temporal resolution. C) Flow-area diagrams were reconstructed to calculate MPA wave speed (pulse wave velocity) by means of linear regression of non-interpolated data points (red color) sampled during early systole. D) Wave intensity spectra were then generated from separated flow and area waveforms. E) Compression or decompression nature of the forward and backward waves was determined from separated area differential waveforms.
First, luminal contours of corresponding magnitude and phase images were segmented using the previously described method applying active contour model, which tracks the edges of the arterial wall initialized by a manual delineation in a single time frame\textsuperscript{58}. Segmented images were then exported into an in-house created made Matlab program (Matlab 2017a, Mathworks, Inc., Natick, MA) for generation of flow and area waveforms which were interpolated using a cubic spline interpolation to achieve 5 millisecond temporal resolution.

Wave propagation speed or pulse wave velocity is classically derived from Bramwell-Hill equation relating the wave propagation speed to distensibility ($D$) and blood density ($\rho$):

\[
c^2 = \frac{1}{\rho D}
\]  

(4)

Distensibility is traditionally calculated as the ratio of the relative area change and pulse pressure reformulating the equation (4) into:

\[
c^2 = \frac{1}{\frac{\Delta A}{\rho \Delta P}} \approx \frac{\Delta P}{\rho dA}
\]  

(5)

However, the calculation of wave propagation speed using pressure derived waveforms is rather non-practical in the clinical setting due its invasiveness. Furthermore, the blood density estimates are usually standardized only to patient specific hematocrit which is not applicable to PAH where compensatory increase in hematocrit can occur due to sub-optimal lung perfusion. The pressure term can be eliminated by incorporating the water-hammer equation:

\[
dP_\pm = \pm \rho c dU
\]  

(6)

where $U$ represent the luminal blood flow velocity. Substitution of the water-hammer relationship into equation (5) results in:

\[
c = \frac{\Delta dU}{dA} = \frac{\Delta Q}{dA}
\]  

(7)
which gives the wave propagation speed in only non-invasive terms obtainable by phase-contrast MRI – flow (Q) waveform and area (A) waveform.

Wave propagation speed analogous to pulse wave velocity in the MPA was then calculated using previously described flow-area method:

\[ c_{MPA} = \frac{dQ}{dA} \]  

(8)

The actual slope of flow-area diagram was calculated by applying linear regression to time points corresponding to early systole. Only non-interpolated early systole data points were applied for this linear regression analysis to minimize the underestimation from incoming wave reflections.

In addition to \( c_{MPA} \), as a non-invasive marker of pulmonary arterial stiffness, we further measured the relative area change defined as previously shown to be calculated as \( \left[\frac{(A_{\text{max}} - A_{\text{min}})}{A_{\text{max}}}\right] \times 100\% \). In order to proceed with the WIA, both area and flow waveforms were separated into their respective forward – positive (travelling from the heart) and backward – negative (travelling from the vasculature) components:

\[ dA_{\pm} = \frac{1}{2} \left( dA \pm \frac{1}{c} dQ \right) \]  

(9)

\[ dQ_{\pm} = \frac{1}{2} \left( dQ \pm cdA \right) \]  

(10)

The net wave intensity was then calculated as product of flow and area waveform differentials:

\[ dl = dAdQ \]  

(11)

Finally, the net wave intensity was separated into its respective forward-positive and backward-negative components as follows:

\[ dl_{\pm} = \pm \frac{c}{4} \left( dA \pm \frac{1}{c} dQ \right) \]  

(12)

In order to classify present intensity waves, respective wave intensity and delta-area waveforms were produced and cross referenced to assure correct interpretation. All positive
waves with a positive $dA_+$ wave were classified as forward compression waves (FCW), whereas all positive waves with negative $dA_+$ were considered as forward decompression waves (FDW). FCW is analogous to right ventricular dP/dt and represents the intensity of ventricular ejection. On the other hand, FDW occurs at the end of systole and is typically associated with diastolic recoil and relaxation. Finally, negative waves with positive $dA_-$ were classified as backward compression waves (BCW), while the negative waves with negative $dA_-$ were described as backward decompression waves (BCD). The units obtained from the non-invasively derived wave intensity give mm$^5$/s which unfortunately represents less intuitive interpretation of the observed wave phenomena than standardly derived pressure-velocity wave intensity providing units of watt/m$^2$. However both measures are qualitatively similar and proportional. All magnitudes of carried wave intensities were indexed to ejected stroke volume as described previously.

*Catheterization*

Right heart catheterization was performed in all patients immediately after MRI acquisition. The cardiac catheterization protocol was performed as recommended by pediatric PAH consensus guidelines. Briefly, catheterization was performed using a 5 or 6-French Swan-Ganz catheter via right internal jugular or right femoral access for measurement of PAH typical metrics including mean pulmonary arterial pressure, RV end-diastolic pressure, indexed pulmonary vascular resistance, and pulmonary arterial wedge pressure. All patients underwent flow evaluation using the Fick principle and patients without a major intracardiac shunt also underwent thermodilution measurement of cardiac output.
Statistical Analysis

Analyses were performed in JMP (version 13.1 or higher; SAS Institute, Cary, NC). Variables were checked for the distributional assumption of normality using normal plots, in addition to Kolmogorov-Smirnov and Shapiro Wilks tests. Variables that were positively skewed (e.g. c-MPA and WIA indices) were natural log-transformed for the correlative analyses.

Demographic and clinical characteristics among children with and without PAH were compared using Student’s t-test for normally distributed continuous variables, Wilcoxon-rank sum test for non-normally distributed variables, and χ² for categorical variables. Additional group comparisons were performed using Kruskal-Wallis or one-way ANOVA tests between the PAH specific WHO-Functional Class groups. Simple linear regression analyses were used to examine association between the WIA indices and typical catheterization / MRI indices of PAH.

All WIA, catheterization, and MRI characteristics were considered for survival univariate analysis. Univariate Cox proportional hazards analysis was applied to assess the predictive ability in all PAH patients. The composite outcome reflective of functional worsening was defined by an escalation in WHO-Functional Class, PAH related hospitalization, need for initiation of prostanoid therapy, syncopal event, or hemoptysis. For variables that were found to be significantly associated with survival univariate analysis, Kaplan-Meier survival curves were constructed with specific log-rank test with the population divided by receiver-operating characteristics to find the most optimal cut-off values. All patients were followed up to the particular event or the end of the study (December, 2018). Significance was based on an α-level of 0.05.
Results

Patient demographics are summarized in Table 1.

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<td>PDE5-i</td>
</tr>
<tr>
<td>ERA</td>
</tr>
<tr>
<td>Prostanoids</td>
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</tbody>
</table>

Data are expressed as mean ± SD or as median with corresponding IQR. WHO = World health organization, IPAH = idiopathic pulmonary arterial hypertension, CHD = congenital heart disease, ASD = atrial septal defect, VSD = ventricular septal defect, PDA = patent ductus arteriosus, CoA = coarctation, PDE5i = phosphodiesterase 5 inhibitors, ERA = endothelin receptor antagonist
We identified 40 children with PAH who underwent same-day MRI and right heart catheterization. There were no significant differences in age, BSA, and sex distribution between PAH subjects and controls (n = 15). At the time of MRI evaluation, 13 children were classified as WHO-Functional Class I, 13 children as WHO-Functional Class II, 12 as WHO-Functional Class III, and 2 children as WHO-Functional Class IV. Twenty patients (50%) were diagnosed with idiopathic PAH, 15 patients (37.5%) had PAH associated with congenital heart disease, and 5 children had PAH due to other causes, including restrictive lung disease (n=3), schistosomiasis (n=1), and hereditary PAH (n=1). The spectrum of congenital heart lesions included atrial septal defect (n=12), ventricular septal defect (n=3), patent ductus arteriosus (n=2), and coarctation of the aorta (n=1). Out of all congenital heart defects, only 2 atrial septal defects were unrepaired with minor shunt (Qp:Qs < 1.2 in both cases). Thirty-eight patients were on phosphodiesterase 5 inhibitors, 28 subjects were receiving endothelin receptor antagonist medications, and 18 patients were on prostanoid therapy.

Cardiac catheterization and MRI hemodynamics are summarized in Table 2 (next page). Median hemodynamic values included mean pulmonary arterial pressure of 42 ± 17 mmHg, PVR index of 16.3 ± 7.6 Woods units, and pulmonary arterial wedge pressure of 8 ± 3 mmHg. Patients with PAH had a median RV end-diastolic pressure of 7 mmHg and elevated RV volumes. Specifically, the median end-diastolic volume index was increased in PAH patients (108 vs. 86 mL/m², P < 0.001) along with increased median end-systolic volume index (52 vs. 40 mL/m², P < 0.001). Patients with PAH had further elevated median indexed stroke volume (53 vs. 45 mL/m², P = 0.012) and mean RV cardiac index (5.0 vs. 3.6 L/min/m², P < 0.001). Lastly, patients with PAH had decreased RV ejection fraction when compared to controls (47 vs. 57 %, P < 0.001).
Table 2. Catheterization and MRI Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>PAH (n=40)</th>
<th>Control (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPAP (mmHg)</td>
<td>42 ± 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVRi (WU.m²)</td>
<td>16.3 ± 7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV EDP (mmHg)</td>
<td>7 (6 - 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAWP (mmHg)</td>
<td>8 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV EDVi (mL/m²)</td>
<td>108 (88 - 131)</td>
<td>86 (76 - 96)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RV ESVi (mL²)</td>
<td>52 (42 - 70)</td>
<td>40 (28 - 42)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RV SVi (mL/m²)</td>
<td>53 (44 - 62)</td>
<td>45 (41 - 51)</td>
<td>0.012</td>
</tr>
<tr>
<td>RVEF (%)</td>
<td>47 ± 11</td>
<td>57 ± 4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RVCI (L/min/m²)</td>
<td>5.0 ± 2.4</td>
<td>3.6 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or as median with corresponding IQR. mPAP = mean pulmonary arterial pressure, PVRi = pulmonary vascular resistance index, RV = right ventricle, EDP = end-diastolic pressure, PAWP = pulmonary arterial wedge pressure, EDVi = end-diastolic volume index, ESVi = end-systolic volume index, SVi = stroke volume index, EF = ejection fraction, CI = cardiac index.

Wave Intensity Analysis

The summary of WIA performed in the MPA is depicted in Table 3 (next page). The median wave speed / pulse wave velocity c-MPA was increased in PAH patients (3.2 vs. 1.6 m/s, \( P < 0.001 \)). Geometric strain derived by mean of relative area change was decreased in PAH patients (29 vs. 42 %, \( P < 0.001 \)). There was no difference in median magnitude of FCW(0) between PAH and controls groups (88 vs. 108 mm²/s/mL, \( P = 0.239 \)). The BCW was present in the mid systole in all PAH patients. Interestingly, seven control subjects revealed mild yet noticeable BCW. The overall median magnitude of BCW(0) was increased in PAH patients (32 vs. 5 mm²/s/mL, \( P < 0.001 \)). We did not observe any prominent BDW in PAH patients nor in controls. Lastly, there was no difference in median magnitude of FDW(0) (32 vs. 28 mm²/s/mL, \( P = 0.856 \)). The comparison of typical PAH patient and control WIA spectra is depicted in Figure 8 (next page).
Table 3. Wave Intensity Analysis

<table>
<thead>
<tr>
<th></th>
<th>PAH (n=40)</th>
<th>Control (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCW(_{(i)}) (mm(^2)/s/mL)</td>
<td>88 (56 - 159)</td>
<td>108 (88 - 222)</td>
<td>0.239</td>
</tr>
<tr>
<td>BCW(_{(i)}) (mm(^2)/s/mL)</td>
<td>32 (22 - 69)</td>
<td>5 (0 - 12)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FDW(_{(i)}) (mm(^2)/s/mL)</td>
<td>32 (18 - 59)</td>
<td>28 (22 - 33)</td>
<td>0.856</td>
</tr>
<tr>
<td>c-MPA (m/s)</td>
<td>3.2 (2.0 - 4.8)</td>
<td>1.6 (1.3 - 2.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RAC (%)</td>
<td>29 ± 11</td>
<td>42 ± 9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or as median with corresponding IQR. FCW\(_{(i)}\) = forward compression wave, BCW\(_{(i)}\) = backward compression wave, FDW\(_{(i)}\) = forward decompression wave, c-MPA = wave speed / pulse wave velocity of the main pulmonary artery, RAC = relative area change.

Figure 8. Comparison of WIA in typical PAH patient (A-C) and control subject with similar age and size (D-F). A) The flow waveform in PAH patient shows characteristic late systolic notch. B) Wave intensity pattern in PAH patient shows prominent backward compression wave (BCW) which positively identified by parallel analysis of separated differential area waveforms. C) In comparison, WIA in control subject does not have backward compression wave E). Furthermore, WIA spectra does not show significant differences between forward compression waveform (FCW) typically associated with dP/dt and RV contraction and similarly forward decompression wave reflective of RV diastolic function appears to have similar magnitude.
In order to understand the nature of pulmonary arterial remodeling between different type of PAH, we compared WIA indices between idiopathic PAH patients and patients with PAH associated with congenital heart disease. The summary of all sub-analyses is depicted in Table 4.

### Table 4. Subgroup Analysis

<table>
<thead>
<tr>
<th></th>
<th>IPAH (n=20)</th>
<th>CHD-PAH (n=15)</th>
<th>P-Value</th>
<th>WHO I+II (n=26)</th>
<th>WHO III+IV (n=14)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCW (i) (mm$^5$/s/mL)</td>
<td>88 (75 - 154)</td>
<td>102 (54 - 165)</td>
<td>0.880</td>
<td>87 (52 - 158)</td>
<td>101 (76 - 152)</td>
<td>0.640</td>
</tr>
<tr>
<td>BCW (i) (mm$^5$/s/mL)</td>
<td>32 (25 - 57)</td>
<td>32 (17 - 72)</td>
<td>0.972</td>
<td>31 (17 - 44)</td>
<td>44 (27 - 110)</td>
<td>0.149</td>
</tr>
<tr>
<td>FDW (i) (mm$^5$/s/mL)</td>
<td>35 (20 - 61)</td>
<td>32 (21 - 56)</td>
<td>0.413</td>
<td>29 (18 - 59)</td>
<td>33 (21 - 50)</td>
<td>0.223</td>
</tr>
<tr>
<td>c-MPA (m/s)</td>
<td>3.3 (2.0 - 4.5)</td>
<td>3.0 (2.5 - 3.7)</td>
<td>0.765</td>
<td>3.0 (2.0 - 4.3)</td>
<td>3.4 (2.2 - 5.6)</td>
<td>0.869</td>
</tr>
</tbody>
</table>

Data are expressed as median with corresponding IQR. FCW$^i$ = forward compression wave, BCW$^i$ = backward compression wave, FDW$^i$ = forward decompression wave, c-MPA = wave speed / pulse wave velocity of the main pulmonary artery.

There were no differences in median FCW$^i$ magnitudes between both considered groups (88 vs. 102 mm$^5$/s/mL, $P = 0.972$), BCW$^i$ (32 vs. 32 mm$^5$/s/mL, $P = 0.880$), FDW$^i$ (35 vs. 32 mm$^5$/s/mL, $P = 0.413$), and c-MPA (3.3 vs. 3.0 m/s, $P = 0.765$). Furthermore, we were investigated whether the WIA indices are associated with present WHO-functional class. We combined groups I + II and III + IV for comparative analysis and found that there are no differences between considered WIA. Specifically, there were no differences in median FCW$^i$ magnitudes (87 vs. 101 mm$^5$/s/mL, $P = 0.640$), BCW$^i$ (29 vs. 33 mm$^5$/s/mL, $P = 0.223$), FDW$^i$ (31 vs. 44 mm$^5$/s/mL, $P = 0.149$), and c-MPA (3.3 vs. 3.0 m/s, $P = 0.869$).

Lastly, in order to investigate the relationship between WIA indices and standard catheterization and MRI hemodynamic indices associated with PAH, we performed simple linear regression analyses between the aforementioned markers. The summary of correlative analyses is depicted in Table 5 (Next page). FCW$^i$ correlated with the indexed end-diastolic (β±SE: 0.007 ± 0.002, $R = 0.42$, $P = 0.013$) and end-systolic volumes (β±SE: 0.007 ± 0.003, $R = 0.42$, $P = 0.014$). BCW$^i$ correlated with mean pulmonary arterial pressure (β±SE: 0.023 ± 0.010, $R = 0.185$).
0.37, \( P = 0.030 \)), indexed end-diastolic volume (\( \beta \pm SE: 0.010 \pm 0.003, R = 0.55, P = 0.001 \)), indexed end-systolic volume (\( \beta \pm SE: 0.010 \pm 0.003, R = 0.53, P = 0.002 \)), and RV ejection fraction (\( \beta \pm SE: -0.038 \pm 0.015, R = 0.41, P = 0.017 \)).

Table 5. Correlative Analysis

<table>
<thead>
<tr>
<th></th>
<th>( \ln (FCW_0) )</th>
<th>( \ln (BCW_0) )</th>
<th>( \ln (c\text{-MPA}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{mPAP} )</td>
<td>-0.010 ( \pm 0.009 ), -0.20, 0.270</td>
<td>0.023 ( \pm 0.010 ), 0.37, 0.030*</td>
<td>0.012 ( \pm 0.006 ), 0.35, 0.043*</td>
</tr>
<tr>
<td>( \text{PVRi} )</td>
<td>-0.013 ( \pm 0.019 ), -0.10, 0.496</td>
<td>0.033 ( \pm 0.021 ), 0.26, 0.138</td>
<td>0.009 ( \pm 0.013 ), 0.10, 0.5103</td>
</tr>
<tr>
<td>( \text{RVEDP} )</td>
<td>0.032 ( \pm 0.058 ), 0.10, 0.5845</td>
<td>0.032 ( \pm 0.058 ), 0.10, 0.584</td>
<td>0.056 ( \pm 0.039 ), 0.24, 0.160</td>
</tr>
<tr>
<td>( \text{EDVi} )</td>
<td>0.007 ( \pm 0.002 ), 0.42, 0.013*</td>
<td>0.010 ( \pm 0.003 ), 0.55, 0.001*</td>
<td>0.004 ( \pm 0.002 ), 0.40, 0.019*</td>
</tr>
<tr>
<td>( \text{ESVi} )</td>
<td>0.007 ( \pm 0.003 ), 0.42, 0.014*</td>
<td>0.010 ( \pm 0.003 ), 0.53, 0.002*</td>
<td>0.004 ( \pm 0.002 ), 0.37, 0.026*</td>
</tr>
<tr>
<td>( \text{EF} )</td>
<td>-0.018 ( \pm 0.014 ), -0.22, 0.214</td>
<td>-0.038 ( \pm 0.015 ), 0.41, 0.017*</td>
<td>-0.024 ( \pm 0.008 ), 0.45, 0.008*</td>
</tr>
</tbody>
</table>

Correlations are expressed as beta values \( \pm SE \) with corresponding \( R \)-value and \( P \)-value. * \( P < 0.05 \), \( \text{mPAP} = \) main pulmonary artery pressure, \( \text{PVRi} = \) pulmonary vascular resistance index, \( \text{RVEDP} = \) right ventricular end-diastolic pressure, \( \text{EDVi} = \) end-diastolic volume index, \( \text{ESVi} = \) end-systolic volume index, \( \text{EF} = \) ejection fraction

Finally, \( c\text{-MPA} \) correlated with mean pulmonary arterial pressure (\( \beta \pm SE: 0.012 \pm 0.006, R = 0.35, P = 0.043 \)), indexed end-diastolic volume (\( \beta \pm SE: 0.004 \pm 0.002, R = 0.40, P = 0.019 \)), indexed end-systolic volume (\( \beta \pm SE: 0.004 \pm 0.002, R = 0.37, P = 0.026 \)), and with RV ejection fraction (\( \beta \pm SE: -0.024 \pm 0.008, 0.45, P = 0.008 \)). Correlations with mean pulmonary arterial pressure and RV ejection fraction are depicted in Figure 9 (Next Page).
Prognostic Analysis

To investigate the prognostic ability of non-invasively derived WIA indices to detect future functional class worsening, we performed univariate proportional hazard analysis between all considered WIA metrics and in comparison, also standard catheterization and MRI PAH specific hemodynamic markers. From all considered patients, we recorded nineteen clinical events indicative of functional worsening including Forest plot summarizing our findings is portrayed in Figure 10 (Next Page). Among the clinical outcomes indicating functional worsening were WHO-functional class escalation (n = 12), syncopal event (n = 3), PAH related hospitalization (n = 2), and a need for initiation of prostanoid therapy (n=2). We found that elevated BCW(i) (HR = 2.91, CI: 1.18 – 7.55, P = 0.019) is prognostic of functional worsening along with reduced FDW(i) (HR = 0.34, CI: 0.11 – 0.90, P = 0.030). Furthermore, increased c-
MPA was prognostic of functional worsening (HR = 3.67, CI: 1.47 – 10.20, \( P = 0.004 \)). In comparison, prognostic catheterization derived indices were mean pulmonary arterial pressure (HR = 4.13, CI: 1.60 – 11.52, \( P = 0.004 \)), indexed pulmonary vascular resistance (HR = 3.84, CI: 1.49 – 11.01, \( P = 0.007 \)), and RV end-diastolic pressure (HR = 2.88, CI: 1.19 – 7.39, \( P = 0.021 \)).

The only MRI hemodynamic index prognostic of functional worsening was RV ejection fraction (HR = 0.28, CI: 0.11 – 0.66, \( P = 0.004 \)). To further illustrate the prognostic potential of non-invasive WIA indices we constructed Kaplan-Meyer curves for WIA metrics which showed to be significant at Cox proportional analysis (Figure 11 – next page). Specifically, BCW\(_{(i)}\)

**Figure 10.** Forest plot summarizing the univariate Cox proportional hazard analysis investigating the prognostic ability of WIA, catheterization, and MRI hemodynamic indices to depict clinical functional worsening. Increased indexed backward compression wave (BCW\(_{(i)}\)) and elevated wave speed / pulse wave velocity measured in the MPA (c-MPA) were prognostic of functional deterioration. Additionally, reduced indexed forward decompression wave (FDW\(_{(i)}\)) was prognostic functional worsening. All typical catheterization markers and MRI derived RV ejection fraction were prognostics of functional worsening.
magnitude $\geq 32 \text{ mm}^2/\text{s/mL}$ was shown to be prognostic of functional worsening along with, along with FDW$_{(i)}$ magnitude $\leq 32 \text{ mm}^2/\text{s/mL}$, and c-MPA $\geq 3.1 \text{ m/s}$.

Figure 11. Kaplan-Meyer curves depicting the prognostic potential of non-invasive WIA metrics.

Discussion

In this study, we have shown that children with PAH have increased wave propagation speed along the MPA (c-MPA) and have incidental systolic BCW (BCW$_{(i)}$), both of which are indicative of elevated pulmonary arterial stiffness. Furthermore, c-MPA and BCW$_{(i)}$ were associated with typical catheterization and MRI indices known to be strongly associated with poor clinical outcomes in both adult and pediatric populations. Most importantly, WIA indices were prognostic of clinical functional worsening suggesting that non-invasive MRI indices should be considered as a component of clinical follow-up in pediatric PAH. Intrinsic pulmonary arterial wall stiffness and backward arterial wave reflections significantly contribute to RV afterload. The more recently appreciated concept of proximal pulmonary vascular remodeling with the strong predictive role of non-invasively measured compliance metrics in adult PAH patients opens a new route of comprehensive PAH analysis using WIA in children.

Wave propagation speed and WIA measurements in the pulmonary circulation using non-invasive imaging are scarce. WIA is typically conducted by means of simultaneous right heart
catheterization and Doppler imaging resulting in pressure-velocity based analysis so called PU method. With regard to incident wave reflection, flow-area method (QA) applied in this study has been previously shown to underestimate wave propagation speed measurements while the PU method typically overestimates them\textsuperscript{63}. However, Quail et al showed that the degree of overestimation for given incident wave is greater by the PU method than degree of underestimation by QA method\textsuperscript{55}. The non-invasive standard for wave speed analysis is the wave propagation method which requires measurement of flow hemodynamic waveform at two different locations sufficiently apart and requires low temporal resolution. Unfortunately, this method is more applicable to the systemic vasculature where sufficient length between aortic segments can be guaranteed and is thus not practical in the pediatric setting per se in the pulmonary vasculature.

\textit{WIA in Pediatric Pulmonary Arterial Hypertension}

The concept of WIA has been widely applied in studies of central aortic stiffness and systemic hypertension\textsuperscript{54,64}. With regard to the pulmonary arterial vasculature, WIA has been typically conducted using combined pressure and velocity waveforms derived from simultaneous right heart catheterization and echocardiography. This approach has been shown to be useful for characterization of adult patients with PAH and chronic thromboembolic pulmonary hypertension\textsuperscript{50,51}, but is less applicable in children with PAH due to its complexity and invasive nature. More recently, Quail et al introduced non-invasive WIA methodology applicable to pulmonary arteries applying a high resolution phase-contrast MRI derived area and flow waveforms to characterize different adult PAH populations\textsuperscript{55}. All aforementioned adult studies described abnormal WIA indices typically characterized by reduced FCW suggestive of diminished RV contractile function, elevated / present BCW indicative of elevated stiffness of
the pulmonary arterial vasculature, and reduced FDW typically associated with reduced RV compliance and poor diastolic function. Furthermore, both invasive and non-invasively derived WIA differentiated between idiopathic PAH and thromboembolic pulmonary hypertension\textsuperscript{51,55}.

In this study, we applied non-invasive phase-contrast MRI to characterize the pulmonary arterial system in children with PAH. In comparison to adult studies, we have not observed significantly reduced FCW and FDW when compared to healthy population of the same age category. This may be partially explained by better RV functional reserve in children when compared to adult PAH patients and different disease mechanisms responsible for vascular remodeling \textsuperscript{2}. The most prominent feature of WIA spectra in our patient population was the presence of BCW which has been previously reported in adult studies and appears to be a characteristic feature of PAH\textsuperscript{50,51,55}. The additive impact of BCW on ventricular afterload has been demonstrated in both systemic and pulmonary circulations and is typically associated with ventricular hypertrophy, eccentric remodeling, and worsening ventricular-vascular coupling ratio \textsuperscript{54,64,65}. BCW represents the primary reflection of the FCW generated by the ventricle and seems to originate primarily in large bifurcations corresponding on the pulmonary side to secondary and tertiary pulmonary artery branches\textsuperscript{66,67}. However, incident wave reflections experienced by the RV do not arise from a single reflection site but instead they are a composition of waves originating from the entire pulmonary arterial tree including distal resistant arterioles. Consequently, it is impossible to exactly determine whether the BCW observed in our study arise predominantly from proximal or distal vessel branches. Serial measurements along the pulmonary arterial tree would have to be conducted to better understand the relationship between experienced incident wave reflections and their respective origins. Previous work by Davies et al applying serial catheterizations and numerical modeling emphasized that reflection sites more
proximal to the location of measurement contribute greatly to the wave magnitude, with distal sites considerably less\textsuperscript{68}. Similarly to the study conducted by Quail et al, we have failed to observe a relationship between WIA markers and steady-state measures (PVRi) reflective of the hemodynamic state of the entire pulmonary arterial system. Overall, these findings would correlate with the current observations highlighting the role of structural remodeling of proximal pulmonary arteries in PAH, as characterized by cellular proliferation and altered extra-cellular matrix composition\textsuperscript{7}. These current and previous findings support the importance of developing novel therapeutic aims targeting proliferative and remodeling pathways in pulmonary arterial endothelial cells rather than treatments focusing on pulmonary vasodilation\textsuperscript{33}.

Elevated wave speed propagation or pulse wave velocity measured in the MPA has been previously described in adult PAH patients\textsuperscript{50,51,55} and in association with RV afterload\textsuperscript{65} but has not previously been described in children with PAH. In this study, we report elevated c-MPA as measured in the mid MPA using a flow-area method that is more sensitive to locally measured stiffness properties. This method might be preferable in children over more conventional wave propagation methods, which require two flow hemodynamic waveforms that are sufficiently temporarily separated from each other\textsuperscript{69}. Furthermore, flow hemodynamic forces acting on endothelial surfaces can promote vascular remodeling through mechanisms that might differ in the MPA and branch pulmonary arteries due to different degrees of stiffness at these locations\textsuperscript{70}. Unfortunately, our imaging protocol did not uniformly apply branch pulmonary artery imaging, which limited our ability to assess the WIA and stiffness evaluation in more distal locations. Future longitudinal studies considering serial WIA evaluations with respect to RV functional evaluation will be required to assess the direct relationship between the ventricular-vascular interplay in PAH.
Abnormal wave reflections with elevated MPA stiffness have been previously associated with RV function in both adult and pediatric PAH populations. Our study showed that FCW, BCW, and c-MPA are each reflective of RV volumes that have been previously associated with mortality in pediatric PAH. The relationship between RV function and pulmonary arterial compliance has been thoroughly evaluated using ventricular-vascular coupling studies, which have been shown to be prognostic of pulmonary vasoreactivity in children with PAH.

However, abnormal wave reflections can significantly contribute to RV afterload and thus perpetuate RV remodeling and dilation, yet the exact pathophysiologic mechanics remains unknown. We speculate that BCW might be decelerating the stroke bolus propagating through proximal pulmonary arteries, which exposes the RV to additional mechanical stress requiring more energetic demand. This would be supported by observed association between RV ejection fraction with BCW and c-MPA. Interestingly, the WIA indices were not associated with PVRi, further suggesting that measured BCW is more reflective of proximal pulmonary arterial stiffening. This additional augmentation of afterload should appear with disease progression when altered flow and pulmonary arterial dilation can induce proximal pulmonary arterial remodeling toward stiffer properties and accelerate the mechanical decoupling of the RV and pulmonary arteries. These findings have been previously described from animal and in vitro studies that demonstrated extensive arterial remodeling in the extracellular matrix as characterized by increased collagen deposition within proximal pulmonary arteries and loss of elastin-dominant behavior during systolic luminal expansion. Altered intrinsic tissue characteristics can reduce the elastic buffering function of “proximal elastic arteries,” leading to altered vessel wall biomechanical properties with abnormal WIA and elevated pulse wave
velocity. These findings suggest that drugs that can inhibit extra-cellular matrix remodeling in large pulmonary arteries may alleviate the increases in afterload with progressive PAH.

_WIA and Functional Worsening_

Functional deterioration is a hallmark of progressive PAH typically requiring an escalation of therapy and more frequent clinical follow-up. The search for novel non-invasive indices prognostic of clinical worsening is of utmost importance to prevent dramatic clinical consequences such as need for atrial septostomy, creation of Pott’s shunt, or lung transplantation. Moledina et al have previously described strong prognostic role of MRI derived typical ventricular functional and volumetric indices toward predicting need for lung transplantation and death in children with PAH. More recently, Swift et al described the most comprehensive non-invasive MRI study in adult PAH patients revealing the independent prognostic roles of RV volumes and relative area change measured in the MPA. These findings suggest that both RV function and proximal pulmonary arterial stiffness may play important roles during clinical follow-up and might be considered part of routine clinical evaluation. In this study, we showed that non-invasively derived WIA indices are predictive of functional worsening in children population which typically proceeds more dramatic clinical outcomes. Specifically, elevated c-MPA and magnitude of BCW have been shown to be associated with functional worsening. We speculate that early non-invasive detection of WIA abnormalities and elevated pulmonary arterial stiffness may lead to therapeutic escalation and aid with the overall clinical management.

Furthermore, we have identified the prognostic role of FDW typically associated with RV compliance and diastolic function. Indeed, previous echocardiographic studies assessing the diastolic strain and strain rate have described a significant prognostic role of diastolic
dysfunction and its related indices in children with PAH\textsuperscript{77,78}. Biventricular myocardial remodeling has been described using delayed gadolinium studies and mechanical deformation analyses in both adult and pediatric PAH population\textsuperscript{79}. The limited ability of a stiff RV to generate sufficient preload leads to elevated systemic venous pressure, abnormal right atrial function, reduced RV stroke volume and decreased pulmonary perfusion. Further evaluation of diastolic dysfunction using more specific ventricular mechanical and flow analysis techniques should be performed to better understand the progressive role of PAH diastolic dysfunction in pediatric PAH patients.

\textit{Limitations}

We acknowledge several limitations associated with our study. First, we did not perform WIA in branch pulmonary arteries that would have enhanced our understanding of the nature of backward wave reflections and possible heterogeneity of proximal pulmonary vascular stiffness. Second, some of our patients were receiving anesthesia or sedation during MRI acquisition that potentially could alter flow hemodynamics. However, as stated previously, this is largely unavoidable because the vast majority of pediatric catheterizations at our institution are performed under general anesthesia, and within this study, only 8 PAH patients (<8 years) required anesthesia for both MRI and catheterization. Another limitation of this study is related to the heterogenous composition of our patient population. While all patients have met hemodynamic criteria for PAH, different disease etiologies were present among our patient population. Future studies will focus on the nature of vascular stiffness and remodeling in specific PAH pathologies. Lastly, our study subjects underwent PC-MRI acquisition on 2 different scanning systems. Ideally, the same acquisition sequence and vendor system should have been applied to every subject. Furthermore,
given that both systems operate on different magnetic field strengths, intersystem variability is an important consideration.

**Conclusion**

We found that children with PAH have abnormal WIA patterns along with elevated pulse wave velocity, which reflect increased proximal pulmonary arterial stiffness. The WIA indices correlated with typical catheterization-derived indices of PVR and were prognostic of clinical functional worsening. Our results suggest that non-invasively derived biomarkers of PVR and stiffness may be helpful during routine clinical follow-up of children with PAH. Furthermore, additional therapeutic targets aimed at proximal pulmonary vascular remodeling should be considered beyond the use of pulmonary vasodilation.
CHAPTER III
MEASURING FLOW HEMODYNAMIC SHEAR STRESS IN CHILDREN WITH PULMONARY ARTERIAL HYPERTENSION

Introduction

Pulmonary arterial hypertension (PAH) in children is a relatively rare and progressive disease with worse survival when compared to adults with PAH. The principal pathophysiologic conditions manifest similarly as in adults, with increasing pressure within the pulmonary vasculature and concomitant stiffening of the proximal pulmonary arteries. Increased afterload on the right ventricle (RV) leads to either adaptive or maladaptive remodeling responses, which are key determinants of long term outcomes. Major limitations persist in improving the clinical care of children with severe PAH due to lack of sensitive, non-invasive measures to accurately assess disease and determine responsiveness to therapeutic interventions. Non-invasive, detailed quantitative characterization of arterial stiffness and mechano-transduction events in the proximal pulmonary arteries could greatly increase our understanding of the progressive pathophysiologic phenomena occurring in pediatric PAH and provide effective strategies to monitor their clinical courses. Furthermore, this will lead to a clearer picture of important differences in pediatric etiology, manifestation, and survival distinct from adult population.

Flow haemodynamic indices of the main pulmonary artery (MPA) in the adult population have been recently investigated using in vitro studies as well as non-invasive imaging methods. Two components play significant roles in vascular maladaptation: wall shear stress and vascular stiffness. Wall shear stress, the primary component of mechanotransduction forces, is markedly decreased in proximal pulmonary conduit arteries. Studies have shown that
altered flow and shear stress are associated with changes in endothelial activation, which include altered cellular signaling and gene expression, as well as endothelial cell geometry \textsuperscript{26,85,34,27}. Increased pulsatility mediated by stiffening of the proximal pulmonary arteries has been associated with progressive increases in the pro-inflammatory response triggered by pulmonary arterial endothelial cells \textsuperscript{76,86}. Furthermore, high pulsatility also alters the vascular response within the medial layer, which causes smooth muscle hypertrophy with parallel changes in protein expression that alter the cellular contractile apparatus and affect vascular stiffness \textsuperscript{87}.

Limited studies of CMR evaluation of the vasculature have been performed in a pediatric PAH population. In a pilot retrospective study of a small population of pediatric PAH patients and only 4 control subjects, we described flow and shear metrics in the right pulmonary artery (RPA), and found results quantitatively similar to adult shear stress investigations \textsuperscript{42}. In this study, we will use phase contrast CMR to show that not only is shear stress significantly altered in proximal vasculature in pediatric PAH in comparison with non-PAH controls, but that this process is intimately tied to changes in vascular stiffness. Furthermore, these vascular parameters are associated with critical changes in the myocardium.

\textbf{Methods}

This study was performed with the approval of the Colorado Multi-Institutional Review Board and all subjects provided written informed consent. We retrospectively identified consecutive PAH patients at Children's Hospital Colorado who have had CMR’s that included short axis stack cine of the ventricles and phase-contrast imaging of the main and right pulmonary artery (MPA and RPA) performed over the period of December 2004 to June 2015. The diagnosis of PAH was established by a prior cardiac catheterization showing a mean
pulmonary arterial pressure (mPAP) of $\geq 25$mmHg and pulmonary vascular resistance of $> 3$ Woods unit x m$^2$. We excluded subjects with pulmonary valve or pulmonary arterial stenosis, or who have had previous surgical intervention on the pulmonary vasculature in order to focus the study on subjects with vascular remodeling due to high resistance and to exclude subjects with anatomic constrictions to flow. All control subjects were prospectively recruited through campus advertisement and were included if they did not have any known cardiac, pulmonary, or systemic disease.

**RV Doppler Echocardiography**

All PAH subjects had standard tissue Doppler echocardiographic studies performed using Vivid 7 ultrasound system (General Electric Medical Systems, Milwaukee, WI) as part of standard of care. Ventricular systolic and diastolic markers were acquired and categorized according to recommendations for imaging in pediatric PAH\textsuperscript{88}. The following standard parameters were collected from the mitral valve and inflow: (1) peak early diastolic filling velocity (E), (2) peak late atrial filling velocity (A), and (3) corresponding E/A ratio. Furthermore, mitral annular Doppler tissue imaging parameters $e'$, tricuspid regurgitation jet, myocardial performance index, and tricuspid systolic to diastolic ratio were recorded in each PAH patient.

**CMR Protocol**

The acquisition protocol was conducted per previously described study protocol\textsuperscript{42}. A gradient echo sequence was applied to obtain tissue intensity and phase velocity maps using a 1.5 Tesla magnet (Magnetom Avanto, Siemens Medical Solutions, Erlangen Germany). Imaging
slices were positioned orthogonal to the vascular lumen. The MPA plane was chosen as the midpoint between the pulmonary valve annulus and the branching point of the branch pulmonary arteries. The RPA plane was taken at midpoint between the bifurcation of the branch pulmonary arteries and the first branching of the RPA (see Figure 13). A typical sequence had a temporal resolution of 14-28 ms, echo times of 2.2-3.5 ms, and a flip angle of 25°. Depending on patient size and field of view, the cross-sectional pixel resolution was 0.82 x 0.82 to 1.56 x 1.56mm with slice thickness of 5 mm. Velocity encoding values were adjusted according to the maximum velocities encountered during scout sequences to avoid aliasing artifact (typical values ranged from 150-200 cm/s).

Figure 13. A) Artistic representation of the specific plane positions for shear stress analysis and summarized final results of $WSS_{sys}$ averaged over all subjects. B) Flow wave comparison between control subject and prominent PAH patient. *$P < 0.0001$, MPA = Main pulmonary artery, RPA = Right pulmonary artery.
Standard short axis images were obtained covering the entire ventricles from the base to the apex\textsuperscript{89}. Post-processing analysis of the CMR images were carried out on QMass (Medis Medical Imaging Systems; Raleigh, NC) in which volume and mass data were manually traced and calculated at end-diastole and end-systole.

\textit{Vascular Parameters}

Both MPA and RPA were segmented over the cardiac cycle using the respective magnitude and phase contrast images for vessel size and wall shear stress analysis, as previously described\textsuperscript{58}. To control for variability among wide age-related differences in pediatric somatic weight and height, vessel diameters were normalized by BSA for inter-group variation and further single variable correlation analysis. Vessel strain was measured by means of relative area change defined as the difference between maximum and minimum area divided by maximum value: \((A_{\text{max}}-A_{\text{min}})/A_{\text{max}}\times 100\%\).

\textit{Ventricular parameters}

Standard RV stroke volume (RVSV), RV cardiac output, and RV ejection fraction (RVEF) were assessed from the short axis stack, then normalized by patient specific BSA values to obtain indexed RVSV and RV cardiac index (RVCI). To include a comprehensive overview of the cardiac and vascular conditions, we also assessed the simplified version of ventriculo-vascular coupling ratio based on CMR-derived volumes, defined as a ratio of RV end-systolic volume and SV\textsuperscript{17}. 
Wall Shear Stress and Flow Analysis

Shear and flow metrics were analyzed by incorporating the time-frame segmented vascular lumens for both proximal pulmonary arteries, as previously described (Matlab Program; Mathworks, Inc., Natick, MA)\(^{45,42}\). Circumferentially averaged axial wall shear stress was measured as peak systolic \((WSS_{sys})\) and time averaged \((WSS_{TA})\). Similarly, peak systolic flow \((Q_{sys})\) and cardiac cycle averaged flow \((Q_{avg})\) values were computed from the respective velocity and phase contrast images. Additionally, the modified version of oscillatory shear index \((OSI)\) previously introduced by Ku was applied to investigate the deviation of shear stress from its principle direction\(^{90,91,46}\).

Measurement Reliability

In a subset of twenty random PAH patients and ten normotensive controls, \(WSS_{sys}\) were determined from two independent segmentations of both MPA and RPA lumens by the same investigator (MS), to assess the intra-observer variability. Further, interobserver error was then computed by two investigator (MS, UT) and computed for \(WSS_{sys}\) of each vessel separately.

Statistical Analysis

All normally distributed group specific data sets are reported as mean with corresponding standard deviations or as median values with interquartile range if non-uniformly distributed. Intergroup significance was assessed using a paired 2-tailed Student t-test in normally distributed data sets and Wilcoxon ranked sum test was applied for non-uniform distributed data. Univariate linear regressional analysis was performed using Spearmen rho method. Statistical analysis was performed using JMP 10.0 (SAS Institute, Cary, NC). Statistical significance was defined as \(P <\)
0.05. The ability of shear metrics to detect the presence of PAH was assessed by receiver operating characteristic (ROC) curves reported with corresponding sensitivity/specificity and Youden Index determined cut-off values.

Results

In this study, we identified 40 PAH patients and recruited 26 age- and BSA- matched controls who met criteria. In all cases, imaging of the MPA provided sufficient quality planar acquisitions for the post processing analysis. Two control subjects and 11 PAH patients were excluded from the RPA analysis due to the presence of artifacts (n=2), poor anatomical positioning (n=7), or noise (n=4) which precludes the ability to define the luminal boundaries for wall segmentation. Group specific demographic data are shown in Table 6.

<table>
<thead>
<tr>
<th>Table 6. Demographic and Basic Hemodynamic Parameters</th>
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</thead>
<tbody>
<tr>
<td>Control (n = 26)</td>
</tr>
<tr>
<td>Age (yrs)</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>BSA (m²)</td>
</tr>
<tr>
<td>WHO I</td>
</tr>
<tr>
<td>WHO II</td>
</tr>
<tr>
<td>mPAP (mmHg)</td>
</tr>
<tr>
<td>PVRi (WU.m²)</td>
</tr>
<tr>
<td>RVSVi (mL/m²)</td>
</tr>
<tr>
<td>RVCI (L/min/m²)</td>
</tr>
<tr>
<td>ESV/SV</td>
</tr>
<tr>
<td>RVEF (%)</td>
</tr>
</tbody>
</table>

Data are expressed as average values ± SD if normally distributed and as median values with corresponding inter-quartile range if non-normally distributed.

There were 23 PAH subjects with World Health Organization (WHO) functional class I and 17 subjects with WHO class II. The average time between the right heart catheterization and CMR acquisition was 236 days (range: 0 to 345 days), and the average time between the echocardiography and CMR was 18 days (range: 0 to 104). The median mPAP and PVRi were
44.5 mmHg and 7.7 Wood units x m², respectively. The echocardiographic subjective evaluation of RV dysfunction revealed 30 patients with normal function, 5 with mild dysfunction, 4 with moderate dysfunction, and 1 with severe RV dysfunction. Two patients had pericardial effusion at the time of the echocardiogram. Echocardiographic data are summarized in Table 7. There were no significant differences between age, BSA, and gender within investigated cohorts.

<table>
<thead>
<tr>
<th>Table 7. RV Echocardiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV systolic function</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Mildly reduced</td>
</tr>
<tr>
<td>Moderately reduced</td>
</tr>
<tr>
<td>Severe reduced</td>
</tr>
<tr>
<td>TAPSE (cm)</td>
</tr>
<tr>
<td>TR Jet</td>
</tr>
<tr>
<td>Pericardial Effusion</td>
</tr>
<tr>
<td>TV E (cm/s)</td>
</tr>
<tr>
<td>TV A (cm/s)</td>
</tr>
<tr>
<td>TV E/A</td>
</tr>
<tr>
<td>TV e' (cm/s)</td>
</tr>
<tr>
<td>TV E/e'</td>
</tr>
<tr>
<td>MPI</td>
</tr>
<tr>
<td>Tricuspid S:D</td>
</tr>
</tbody>
</table>

Data are expressed as average values ± SD.
TAPSE = tricuspid annular planar systolic excursion, TR = Tricuspid Regurgitation, TV = Tricuspid valve, MPI = myocardial performance index.

Several CMR parameters were significantly different between PAH and control groups: RVEF was lower in PAH patients (44% vs. 57%, \( P < 0.0001 \)) and right ventricular cardiac index was increased in PAH group (3.9ml/min/m² vs. 3.5ml/min/m², \( P < 0.0018 \)). The ventricular vascular coupling ratio was dramatically higher in the PAH group. The components of this ratio represent vascular afterload to ventricular contractility; thus, a higher ratio may signal a trend a state whereby the rise in vascular afterload outpaces the compensatory increase of ventricular contractility. Normalized vessel diameter was larger in both MPA and RPA in the PAH group (\( P \))
< 0.05 and \( P < 0.0001 \)). Finally, vascular strain was reduced in the PAH group in both the MPA and RPA \( (P < 0.0001 \) and \( P < 0.05 \)).

All phase-contrast CMR derived hemodynamic and flow measures are shown in Table 8.

### Table 8. CMR Derived Hemodynamic Measurements

<table>
<thead>
<tr>
<th></th>
<th>Main Pulmonary Artery</th>
<th>Right Pulmonary Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 26)</td>
<td>PAH (n = 40)</td>
</tr>
<tr>
<td>WSS\textsubscript{sys} ((\text{dyne/cm}^2))</td>
<td>6.49 ± 1.74</td>
<td>4.37 ± 2.36*†</td>
</tr>
<tr>
<td>WSS\textsubscript{TA} ((\text{dyne/cm}^2))</td>
<td>1.89 ± 0.52</td>
<td>1.04 ± 0.66*†</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td>2.59 ± 0.27</td>
<td>3.34 ± 0.83*†</td>
</tr>
<tr>
<td>(D\textsubscript{norm} ,(\text{cm/m}^2))</td>
<td>1.84 ± 0.66</td>
<td>2.73 ± 1.23*</td>
</tr>
<tr>
<td>(Q\textsubscript{Peak} ,(\text{L/min}))</td>
<td>16.7 ± 4.3</td>
<td>19.6 ± 8.0</td>
</tr>
<tr>
<td>(Q\textsubscript{Avg} ,(\text{L/min}))</td>
<td>4.1 ± 1.0</td>
<td>4.9 ± 2.3</td>
</tr>
<tr>
<td>Strain (%)</td>
<td>36 ± 9</td>
<td>25 ± 10*†</td>
</tr>
<tr>
<td>OSI (%)</td>
<td>0.17 (0.28 - 1.13)</td>
<td>0.46 (0.11 - 0.87)*</td>
</tr>
<tr>
<td>(V\textsubscript{max} ,(\text{cm/s}))</td>
<td>86.7 ± 18.0</td>
<td>85.3 ± 24.4</td>
</tr>
<tr>
<td>(V\textsubscript{avg} ,(\text{cm/s}))</td>
<td>55.97 ± 11.0</td>
<td>46.5 ± 19.5</td>
</tr>
</tbody>
</table>

Data are expressed as averages ± SD or medians with inter-quantile range.*\( P < 0.05 \), †\( P < 0.0001 \)

\( WSS\textsubscript{sys} \) and \( WSS\textsubscript{TA} \) was strongly decreased in the PAH group in both vessels (all \( P < 0.0001 \)). Peak flow metrics did not differ in analyzed segments, except \( Q\textsubscript{avg} \), which was significantly larger in the RPA of PAH group. The OSI was significantly larger in PAH in the MPA \( (P < 0.05) \) but not in the RPA. The peak velocity \( V\textsubscript{max} \) measured through the described planes was significantly higher in the RPA of controls compared to PAH subjects (104.5 cm/s vs. 83.2 cm/s, \( P < 0.05 \)), but interestingly this difference was not present in the MPA. Similarly, the average velocity \( V\textsubscript{avg} \) was significantly higher in both groups in the RPA as well. The phase contrast CMR derived haemodynamic metrics did not show significant differences between the PAH WHO I and WHO II subgroups. There was 3.06% intra-observer variability in computation of \( WSS\textsubscript{sys} \) in the MPA and 2.24% in the RPA. Inter-observer variability assessed directly from the segmentation process of both vessels was 2.19% in the MPA and 1.76% in the RPA.
The $WSS_{sys}$ evaluated in the MPA revealed nearly linear positive correlation with vascular strain ($r = 0.63, P < 0.0001$). A negative correlation was found between $WSS_{sys}$ and diameter measured in MPA ($r = -0.62, P < 0.0001$). Importantly, the MPA $WSS_{sys}$ also displayed a positive logarithmic association with RVEF ($r = 0.65, P < 0.0001$) (see Figures 14-16).

**Figure 14.** Regression analysis of $WSS_{sys}$ and strain measured at the MPA cross section. The trend revealed significant positive nearly linear relationship between two metrics suggesting that endothelial shear stress may be the further potentiating agent of stiffness.

**Figure 15.** Regression analysis of $WSS_{sys}$ and MPA size showing a significant negative relationship between the two metrics. The negative curvilinear relationship reflects the Haagen-Poiseuille relationship, which describes an inversely proportional relationship between vessel diameter and the wall shear stress.
Strain in the MPA also positively correlated logarithmically with RVEF ($r = 0.63$, $P < 0.0001$). These trends existed between the RPA and measured markers as well but without statistical significance. Additionally, the $WSS_{sys}$ measured in both vascular segments significantly correlated with the PVRi in negative exponential fashion. The regression analysis in MPA ($r = -0.66$, $P < 0.001$) was more significant than in RPA ($r = -0.59$, $P < 0.01$) (Figure 17).

**Figure 16.** Regression analysis of $WSS_{sys}$ with the preload dependent marker, right ventricular ejection fraction. A positive logarithmic trend between two markers showed a significant relationship. Based on observed trend between strain and the $WSS_{sys}$, this relation suggests that cardiac systolic performance is affected by stiffening of the proximal pulmonary vasculature as well.

Strain in the MPA also positively correlated logarithmically with RVEF ($r = 0.63$, $P < 0.0001$). These trends existed between the RPA and measured markers as well but without statistical significance. Additionally, the $WSS_{sys}$ measured in both vascular segments significantly correlated with the PVRi in negative exponential fashion. The regression analysis in MPA ($r = -0.66$, $P < 0.001$) was more significant than in RPA ($r = -0.59$, $P < 0.01$) (Figure 17).

**Figure 17.** Regression analysis of $WSS_{sys}$ with right heart catheterization derived PVRi in MPA A) and RPA B). Both trends showed significant negative exponential relationships despite the considerable mean period (236 days) between catheterization and CMR evaluation. Average $WSS_{sys}$ values of PVRi tertiles in the MPA C) and RPA D). There was not a significant inter-tertile difference between vessels.
There was no significant correlation with the mPAP and shear metrics in either vessel segments. Lastly, a significant positive linear correlation existed between TAPSE and the MPA $WSS_{sys}$ ($r = 0.66, P < 0.001$) (Figure 18). No further correlation was found between echocardiographic markers and CMR-derived vascular parameters.

**Figure 18.** The correlation between echocardiographically derived TAPSE and the MPA $WSS_{sys}$. The observed relationship implies an association between RV systolic performance and the measured shear metrics in the proximal pulmonary vasculature.

ROC curve analysis for $WSS_{sys}$ in both vessels (Figure 19) were generated in order to assess the ability to detect PAH in pediatric patients.

**Figure 19.** The receiver operating characteristic curves of $WSS_{sys}$ were constructed in order to detect pulmonary arterial hypertension in MPA A) and RPA B).
With the cut-off value 4.5 dyne/cm$^2$ and ROC = 79.1%, $WSS_{sys}$ in the MPA showed moderate sensitivity (67.5%) and high specificity (84.6%). Similarly in the RPA, the cut-off value 8.5 dyne/cm$^2$ and ROC = 81.1%, $WSS_{sys}$ showed moderate sensitivity (71.4%) and high specificity (95.8%). The OSI measured in the MPA and RPA both showed ROC < 25% and with non-significant sensitivity/specificity.

**Discussion**

Our current study reports marked decreases in $WSS_{sys}$ and $WSS_{TA}$ metrics in the proximal pulmonary arteries of pediatric subjects with PAH, providing additional support and extension of our past observations$^{42}$. Our findings are also supportive of those from a multicenter study conducted by Barker *et al.* in an adult PAH population$^{48}$. Additionally, we found marked associations between RV systolic function and shear stress, and that the PAH population in this study manifested increased vascular size. The overall wall shear stress values for both investigated pediatric groups are two fold higher than in adult pulmonary hypertension population$^{40,48}$. These changes are mainly due to principal vessel geometric and cardiac performance differences observed in the pediatric population compared to adults. Furthermore, the trend toward high shear stress values between this study and past adult studies might be due to higher resolution scans acquired by our protocol.

More importantly, $WSS_{sys}$ measured in the MPA correlated positively with vascular strain, which is a well-validated marker of vascular stiffness$^{92,93}$. In our study, vascular strain was significantly reduced in both MPA and RPA in the PAH group, in agreement with previous studies of this parameter in adults$^{61}$. The negative correlation with the MPA diameter follows the simplified wall shear stress definition described by Haagen-Poiseuille, where the shear stress is
inversely proportional to cubic power of vessel diameter\textsuperscript{45,94}. We speculate that the effect of progressive dilation is the principle mechanism for reduced $WSS_{sys}$ since there was no significant variance in flow metrics. However, given the pulsatile nature of proximal flow, the application of steady flow theory to explain these findings should be used with caution.

Throughout the literature, wall shear has been shown to play a major role in endothelial function and vascular tissue response\textsuperscript{95}. While the majority of these studies have been focused on the systemic circulation, the functional changes within the pulmonary endothelial lining are no less important and exhibit similar shear stress dependent changes.\textsuperscript{96} We believe that dilation of proximal conduit vessels decreases wall shear stress in order to maintain flow. Wall shear, in turn may be the principle driving mechanism through which mechanotransduction promotes vascular stiffness. While proximal arterial segments were significantly dilated in our PAH group, RVSVi remained unchanged when compared to controls. Contrast this to adult PAH populations, in which RVSVi is decreased and tends to correlate with measured shear metrics. Further evidence supporting the presence of proximal vascular stiffness can be observed by detailed analysis of the flow profile through MPA and RPA. The flow wave in PAH patients presented characteristics of less compliant vessel including increased rate in flow during initial upstroke in systole and presence of mid-systolic notch (Figure 1). Finally, the $WSS_{sys}$ measured in considered vascular segments, correlated significantly with PVRi\textsuperscript{19,97}. This phenomena has never been shown in children.

Interestingly, the majority of our PAH patients showed a trend towards an uncoupled RV-pulmonary artery axis with a ventricular-vascular coupling ratio higher than 1.0. However, the higher RVCI and peak systolic flow $Q_{sys}$ in the PAH group suggests that the RV contractility is still responding within its reserve. The average RVCI value of our PAH group is in agreement
with large registry based study indicating preserved RV function in pediatric patients\textsuperscript{6}. It was speculated before that unlike the adult population, RVCI is stable in pediatric patients or increased as part of ventricular remodeling despite tremendously increased afterload\textsuperscript{2}. The RVEF, unlike RVCI, did correlate positively with RAC and $WSS_{sys}$ suggesting that latter measured metrics are surrogate markers of stiffness, affecting cardiac contractile condition rather than RV energetic performance. The curvilinear relationship between the RVEF and $WSS_{sys}$ measured in the MPA implies that shear may be severely reduced in advanced disease stage due to reduced flow and vascular dilation.

Directional changes in shear stress over a cardiac cycle as assessed by OSI can contribute to endothelial dysfunction and to intimal fibrosis, which further augments vascular stiffening. In this study, we show an increased OSI in the MPA of PAH patients, however, this difference was not observed in the RPA. An increase in OSI has also been shown in other vasculopathies associated with intimal hyperplasia, including coronary and cerebral arteries\textsuperscript{98,99}. We speculate that the MPA is more prone to wall shear directional deviations due to close proximity to the pulmonic valve and proclivity to generate rigorous vortical flow patterns. As previously shown, vortex-like structures within the cardiovascular system comprise a specific set of physiologic flow patterns and wall shear information required for complete three dimensional domain analysis\textsuperscript{100}. Oscillatory flow and reduced endothelial shear both have pathogenic effects possibly resulting in pro-inflammatory and pro-thrombotic events\textsuperscript{26,96}. Distal vasculature is more prone to aforementioned changes resulting in an overall heightened vasoconstrictive/stiffness state, but in this study we have been able to also show decreased wall shear stress in pediatric pulmonary hypertension, measured in proximal pulmonary conduit and simultaneously correlate shear metrics to stiffness markers.
Study Limitations

One potential limitation of this study is that our subject population represents a relatively healthy group of children with PAH, as reflected in the fact that no subject had a WHO functional class greater than II. However, finding differences among this population suggests greater sensitivity and the potential utility of these measures even in milder disease. Another limitation of this study was our assumption of a uniform viscosity. The product of shear rate and viscosity serves as wall shear stress computation within the software algorithm. However, a patient-specific viscosity approach has been previously successfully used in many systemic and pulmonary shear stress studies\(^{48,101}\).

An additional limitation is the mean time between CMR and catheterization is large for our PAH group. Future research will require performance of both studies within a short period. We included catheterization data here in order to initiate the discussion that CMR-derived parameters may be powerful surrogates of conventional catheterization-derived data. This is particularly important to explore, since catheterization is the standard of care, but exposes children to repeated radiation, anesthesia, and complications associated with intracardiac catheter manipulation.

Conclusions

The wall shear stress is reduced in the proximal pulmonary arteries of pediatric PAH patients concomitantly with vascular strain, indicating stiffness within the proximal pulmonary vasculature. These results were also followed by increased OSI in MPA, which further has a potential to accelerate stiffness process. Furthermore, these vascular parameters have important association with RV myocardial function. The vascular characterization in proximal pulmonary
conduit could provide important insights to pathogenesis and therapeutic effects in pediatric PAH population.
CHAPTER IV

RELATIONSHIP BETWEEN SHEAR MEDIATED FORCES AND PULMONARY VASCULAR STIFFNESS

Introduction

Proximal pulmonary vascular stiffness and abnormal flow hemodynamics are increasingly recognized as being strongly associated with elevated right ventricular (RV) afterload in both children and adults with pulmonary arterial hypertension (PAH).\textsuperscript{22,33,102–104} Non-invasive surrogate indices of main pulmonary arterial (MPA) stiffness have previously been shown to reflect disease severity, catheterization-derived hemodynamics, and most importantly, poor clinical outcomes in heterogeneous adult PAH populations.\textsuperscript{62,72,82} However, the prognostic utility of indices of MPA stiffness in pediatric PAH is unknown. Furthermore, the exact interplay between proximal pulmonary vascular stiffness and abnormal flow hemodynamics in the proximal pulmonary arterial conduit is still unknown despite increasing evidence supporting a key role of flow-mediated vascular remodeling in disease progression of the pulmonary and systemic circulations.\textsuperscript{32,35}

Characterization of pulmonary vascular stiffness in children is a challenging task due to the invasiveness of right heart catheterization, heterogeneity of congenital heart defects (CHD) commonly associated with pediatric PAH, and limited number of studies that follow pediatric patients through transition to adult PAH programs.\textsuperscript{2,5} While not direct measurements of intrinsic vascular properties, non-invasive markers of pulmonary vascular stiffness may obviate some of these challenges. Using non-invasive cardiac magnetic resonance (CMR), we have previously reported that reduced relative area change (RAC) and hemodynamic wall shear stress (WSS) in large pulmonary arteries are strongly associated with disease severity in children.\textsuperscript{42,105} Elevated
WSS is typically associated with systemic vasculopathies, and consequently with endothelial damage and extracellular matrix degradation\textsuperscript{27,35}. However, decreased WSS is commonly present in the proximal pulmonary arteries of patients with PAH, which likely promotes a pro-inflammatory endothelial cell phenotype, smooth muscle cell proliferation, and inflammatory cell infiltration\textsuperscript{26,86}. Further dilation of proximal pulmonary vasculature leads to elevated stiffness, and consequently to additional rise in RV afterload.

To more comprehensively investigate this pathophysiologic process in children with PAH, we evaluated serial changes in pulmonary flow hemodynamics and MPA stiffness and sought to determine the clinically prognostic potential of MPA stiffness indices. We hypothesized that non-invasively derived markers of vascular stiffness, pulse wave velocity (PWV) and RAC, will change, in a longitudinal fashion, with shear hemodynamics and will predict clinical outcomes in children with PAH. Better understanding of the interplay between flow hemodynamics and vascular stiffness with respect to clinical prognostics may provide more information to guide therapeutic management and pharmacological targeting of PAH.

**Methods**

This study was approved by the Colorado Multi-Institutional Review Board, and all subjects provided written informed consent. Patients with PAH seen by the Pulmonary Hypertension Clinic at Children’s Hospital Colorado who underwent comprehensive cardiac magnetic resonance (CMR) from December 2007 to August 2016 were included. The initial diagnosis of PAH was established after evaluation by our Pulmonary Hypertension Program, which included echocardiograms and a prior cardiac catheterization, according to accepted guidelines\textsuperscript{22,23}. World Health Organization functional class (WHO-FC) was evaluated during the
initial visit and all subsequent clinical visits at the Pulmonary Hypertension clinic with the time interval between individual follow-ups ranging between 3 weeks to 6 months. Exclusion criteria included 1) present pulmonary valve or pulmonary arterial stenosis determined by CMR or echocardiography, 2) patients with absent primary pulmonary branch, and 3) previous surgical intervention on the pulmonary vasculature involving artificial material, such as right ventricular to pulmonary arterial conduits. Control subjects were prospectively recruited through campus advertisement and were included if they did not have any known underlying cardiac, pulmonary, or systemic disease. Catheterization-derived data from the chronologically closest study to the CMR was used for this study.

**CMR Acquisition**

The acquisition protocol was performed as previously described\(^{42,105}\). A gradient echo ECG gated sequence was applied to obtain tissue intensity and phase velocity maps using a 1.5 or 3.0 Tesla magnet (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany; Ingenia, Philips Medical Systems, Best, The Netherlands). The flow hemodynamic parameters were measured using phase-contrast CMR, in the mid-section of the MPA in orthogonal fashion confirmed by corresponding angiogram (Figure 20, next page). The free breathing phase-contrast CMR with Cartesian encoding and retrospective sorting had a temporal resolution of 14–28 ms with 30 to 50 phases, echo times of 2.2–3.5 ms, matrix: 160 × 256, flip angle of 25° with 100% of the k-space sampled without any further temporal resolution reconstruction algorithms being applied. Depending on patient size and field of view (128-225 × 210-360 mm), the cross-sectional pixel resolution was 0.82 × 0.82–1.56 ×1.56 mm\(^2\) with a slice thickness of 5 mm. Resulting acquisition time varied between 2 to 3 minutes, depending upon heart rate. Velocity
encoding values were adjusted according to the maximum velocities encountered during scout sequences to avoid aliasing artifact (typical values ranged from 100 to 150 cm/s) per consensus recommendation\textsuperscript{43}.

Right ventricular dimensional analysis was performed through standard short-axis images with coverage of the ventricles from base to apex as described previously\textsuperscript{105}. RV volume and cardiac output were indexed for body surface area (BSA). In addition to standard RV volumetric and functional metrics we calculated RV ventricular-vascular coupling ratio (VVCR) using simplified CMR method (end-systolic volume/stroke volume)\textsuperscript{17}.

\textbf{Figure 20.} A) Magnetic resonance angiography reconstructed MPA with superimposed plane of phase-contrast CMR was applied to ensure the universally applied location for flow and stiffness analysis. B) Exemplary phase-contrast image with the segmentation line derived from corresponding magnitude image. C) Magnitude image with location specific labeled point of WSS analysis. D) Created flow and area waveforms with highlighted region of the PWV analysis at the early ejection phase.
**Pulmonary Vascular Stiffness Analysis**

To characterize MPA stiffness, we used measures of flow-area (dQ/dA) that are applicable for proximal regions of great vessels, as previously described\(^{55,106}\). Flow and area change waveforms were analyzed from time-frame segmented respective phase-contrast and magnitude images (Matlab Program; Mathworks, Inc., Natick, MA, USA). To limit the effect from backward wave reflections, we sampled data points for PWV evaluation only during the early phase of ejection and excluded data points suggestive of early reflection which would have created an upstroke notch within the flow curve or when the flow-area curve revealed a plateau at the end of the upstroke phase (Figure 20). For the final computation of dQ/dA slope representing PWV, 4 to 6 data points were needed to ensure reliable linear fit\(^{55,69,106}\). The RAC was computed from the segmented magnitude images as \((A_{\text{max}} - A_{\text{min}})/A_{\text{max}} \times 100\%\).

**Shear Hemodynamics**

The through-plane WSS at the MPA plane was computed as shown previously from 8 points along the MPA lumen\(^{42,106}\). Specifically, WSS was sampled at 45° increments along the MPA lumen for localized position specific analysis. These positions were denoted to reflect anatomical location within the MPA plane as S, LS, RS, L, R, RI, LI, and I, representing the superior, left-superior, right-superior, left, right, right-inferior, left-inferior, and inferior MPA lumen locations (Figure 20). From the WSS waveform, maximum systolic WSS (WSS\(_{\text{max}}\)), time-averaged WSS (WSS\(_{\text{TA}}\)), and oscillatory shear index (OSI) were collected. OSI, previously described by Ku et al is descriptive of the deviation of shear stress from its principle direction\(^{107}\). The OSI describes the directional uniformity of WSS which can be comprised particularly in the presence of chaotic and turbulent flow. Highly oscillatory shear described by high OSI values is
associated with poor endothelial function and vascular remodeling. Additional parameters were sampled from patient specific flow waveforms: maximum flow ($Q_{\text{max}}$), maximum velocity ($V_{\text{max}}$), maximum systolic diameter ($D_{\text{max}}$).

**Statistical analysis**

Analyses were performed in JMP (version 13.1 or higher; SAS Institute, Cary, NC). Variables were checked for the distributional assumption of normality using normal plots, in addition to Kolmogorov-Smirnov and Shapiro Wilks tests. Variables that were positively skewed (e.g. PWV, oscillatory shear index, VVCR) were natural log-transformed for the correlative analyses. Demographic and clinical characteristics among children with and without PAH were compared using student $t$-test for normally distributed continuous variables, Wilcoxon-rank sum test for non-normally distributed variables, and $\chi^2$ for categorical variables. Additional group comparisons were performed using Kruskal-Wallis or one-way ANOVA tests between the PAH specific WHO-FC groups and PAH clinical categories. Generalized linear regression models were used to examine association between the MPA stiffness (PWV and RAC) and RV volumetric and functional indices and were adjusted for age, BSA and sex.

To investigate the relationship between WSS and MPA stiffness over time, a random coefficient model with an intercept and slope fit for each patient with at least two CMR measurements was used as described previously. In addition, a bivariate version of the random coefficients model was applied to investigate whether the trends in WSS over time are associated with changes of MPA stiffness indices. This modeling approach is achieved by simultaneously fitting two univariate mixed effects models, one for each outcome, and
specifying a joint multivariate distribution on the random effects. A benefit of this method is that it does not require the outcomes to be measured at the same time.

All MPA and RV characteristics were considered for survival univariate analysis. Univariate Cox proportional hazards analysis was applied to assess the predictive ability of RV and MPA specific variables in all 57 PH patients. Two types of composite outcomes were considered for prognostic analysis. Composite severe outcomes were defined as death, lung transplantation, initiation of intravenous or subcutaneous prostacyclin therapy, clinically indicated atrial septostomy or need for a Pott’s shunt. Composite moderate outcomes were defined by an escalation in WHO-FC, PAH related hospitalization, syncopal event, or hemoptysis. For variables that were found to be significantly associated with survival univariate analysis, Kaplan-Meier survival curves were constructed with specific log-rank test with the population divided by receiver-operating characteristics to find the most optimal cut-off values. All patients were followed up to the particular event or the end of the study (August, 2016). Significance was based on an α-level of 0.05.

Results

Comprehensive patient characteristics and baseline hemodynamics are summarized in Table 9 (next page). Fifty-seven patients (average age, 12.1 ± 5.5 years; range: 1.5 to 18.1 years) with baseline CMR acquisition underwent successful MPA stiffness and shear evaluation, and 21 patients had two or more follow-up CMR acquisitions at a mean interval of 3.5 years (range: 0.6-6 years). Twenty-three patients had idiopathic PAH, 22 patients had PAH associated with CHD, and 14 had PAH due to other causes. Specific CHD lesions included atrial septal defect (n = 7), ventricular septal defect (n = 6), atrioventricular septal defect (n = 2), coarctation of the aorta (n
= 2), patent ductus arteriosus (n = 3), partially anomalous pulmonary venous return (n = 1), transposition of great arteries (n = 1), and pulmonary vein stenosis (n = 1) with all defects being post repair. Twelve patients were classified within WHO-FC I, 29 subjects as WHO-FC II, 11 subjects as WHO-FC III, and 6 subjects with WHO-FC IV.

| Table 9. Patient characteristics & Baseline hemodynamics |
|---------------------------------|----------------|----------------|
| **PAH (n = 57)** | **Control (n = 23)** | **P value** |
| Age (yrs) | 12.1 ± 5.5 | 13.1 ± 3.7 | 0.4021 |
| Sex | 54% | 52% | 0.5136 |
| BSA (m²) | 1.28 ± 0.42 | 1.35 ± 0.30 | 0.4248 |
| WHO-FC | | | |
| I | 12 (21%) | | |
| II | 29 (51%) | | |
| III | 11 (19%) | | |
| IV | 6 (10%) | | |
| IPAH | 23 (40%) | | |
| CHD | 22 (38%) | | |
| Others | 14 (24%) | | |
| PDE5i | 40 (70%) | | |
| ERA | 26 (46%) | | |
| Prostacyclin | 25 (44%) | | |
| mPAP (mmHg) | 44 ± 17 | | |
| PVRi (WU.m²) | 9 ± 6 | | |
| RVEDVi (mL/m²) | 122 ± 47 | 87 ± 13 | < 0.0001 |
| RVESVi (mL/m²) | 69 ± 45 | 37 ± 8 | < 0.0001 |
| SVi (mL/m²) | 53 ± 14 | 51 ± 8 | 0.4726 |
| EF (%) | 46 ± 12 | 58 ± 5 | < 0.0001 |
| VVCR | 1.33 ± 0.75 | 0.74 ± 0.16 | 0.0005 |
| CI (l/min/m²) | 4.1 ± 1.8 | 3.6 ± 0.8 | 0.1241 |
| HR | 74 ± 24 | 70 ± 10 | 0.3819 |

Data are reported as mean ± SD. WHO-FC = world health organization – functional class, IPAH = idiopathic pulmonary hypertension, CHD = congenital heart disease, PDE5i = phosphodiesterase-5 inhibitors, ERA = endothelin receptor antagonists, mPAP = mean pulmonary arterial pressure, PVRi = pulmonary vascular resistance index, EDVi = end-diastolic volume index, ESVi = end-systolic volume index, SVi = stroke volume index, VVCR = ventricular-vascular coupling ratio, EF = ejection fraction, CI = cardiac index, HR = heart rate

At the time of initial CMR acquisition, all patients were receiving PAH therapy (40 phosphodiesterase-5 inhibitors, 26 endothelin receptor antagonists, and 25 prostanoid derivatives). 28 patients were receiving monotherapy, and 16 had dual combination therapy, and
13 were receiving triple combination therapy. Catheterization-derived mean pulmonary arterial pressure (PAP) was 44 ± 17 mmHg with a pulmonary vascular resistance index (PVRI) of 9 ± 6 Wood units*m². The median time between catheterization and CMR acquisition was 132 days (range: 0 – 218 days). Comparison of standard RV volumetric measures between PAH and control populations revealed significantly elevated indexed end-diastolic and end-systolic volumes in the PAH group (both P < 0.0001). Furthermore, the RV ejection fraction was significantly decreased in PAH group (P < 0.0001) whereas the VVCR was significantly elevated (P = 0.0005) in the same group. Indexed stroke volume, cardiac index, and heart rate were similar between PAH and control groups.

**Baseline MPA Stiffness and WSS Analysis**

The hemodynamic and MPA stiffness analysis is summarized in Table 10.

<table>
<thead>
<tr>
<th></th>
<th>PAH (n = 57)</th>
<th>Control (n = 23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSS&lt;sub&gt;max&lt;/sub&gt; (dyne/cm²)</td>
<td>4.2 ± 2.2</td>
<td>6.3 ± 1.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>WSS&lt;sub&gt;TA&lt;/sub&gt; (dyne/cm²)</td>
<td>0.9 ± 0.5</td>
<td>1.8 ± 0.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>OSI</td>
<td>0.020 (0.017 – 0.067)</td>
<td>0.007 (0.001 – 0.010)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Q&lt;sub&gt;max&lt;/sub&gt; (L/min)</td>
<td>17.2 ± 7.2</td>
<td>15.2 ± 3.7</td>
<td>0.1577</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (cm/s)</td>
<td>80 ± 29</td>
<td>79 ± 18</td>
<td>0.886</td>
</tr>
<tr>
<td>D&lt;sub&gt;max&lt;/sub&gt; (cm)</td>
<td>3.1 ± 0.6</td>
<td>2.6 ± 0.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D&lt;sub&gt;min&lt;/sub&gt; (cm)</td>
<td>2.8 ± 0.6</td>
<td>2.0 ± 0.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RAC (%)</td>
<td>25 ± 11</td>
<td>37 ± 9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>2.8 (1.9 - 4.2)</td>
<td>1.4 (1.3 - 1.8)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD or median with interquantile range. WSS<sub>max</sub> = maximum systolic wall shear stress, WSS<sub>TA</sub> = time-averaged wall shear stress, OSI = oscillatory shear index, Q<sub>max</sub> = maximum flow, V<sub>max</sub> = maximum velocity, D<sub>max</sub> = maximum diameter, RAC = relative area change, PWV = pulse wave velocity

The baseline PWV was significantly increased in the PAH group (2.8 m/s vs. 1.4 m/s, P < 0.0001) and correspondingly RAC was significantly depressed in the same group (25% vs.
Representative PWV analysis with corresponding flow and area waveforms is depicted in Figure 21 (next page).

**Figure 21.** A) Representative control flow hemodynamic waveform with corresponding PWV analysis depicted by linear fit between the flow and area data points during systolic upstroke. B) Representative PAH case depicts typical characteristics of MPA stiffness, including presence of downstroke notch and backward post-systolic flow.

There was no intergroup variability in PWV between different WHO-FC groups (Figure 22A). Similarly, we did not observe any differences in PWV between APAH-CHD or IPAH (Figure 22B).

**Figure 22.** A) Intergroup analysis between different WHO-FC categories did not reveal any variability between considered groups. B) Similarly, no intergroup variability existed among different PAH categories. $P$ values are derived Kruskal-Wallis analysis.
Baseline shear hemodynamic analysis revealed decreased $WSS_{\text{max}}$ and $WSS_{\text{TA}}$ in PAH patients (both $P < 0.0001$). The oscillatory shear index was significantly increased in the PAH population ($P = 0.0001$). Regional $WSS_{\text{max}}$ distribution along the MPA lumen for control and PAH is depicted in Figure 4.

![Figure 4](image)

**Figure 4.** Regional $WSS_{\text{max}}$ distribution comparison between controls (blue dashed line) and PAH group (red line). The $WSS_{\text{max}}$ was significantly lowered along the entire aspect of the MPA lumen except at the inferior portion of the vessel.

The $WSS_{\text{max}}$ was significantly decreased in all considered anatomical points, except at the inferior aspect of the MPA. The major WSS determinants $Q_{\text{max}}$, $V_{\text{max}}$, and $D_{\text{max}}$ revealed variability between PAH and control groups only in $D_{\text{max}}$ (3.1 cm vs. 2.6 cm, $P < 0.0001$). The baseline correlations between stiffness markers (PWV and RAC) and RV volumetric and functional indices are summarized in Table 11. Both PWV and RAC correlated significantly with ejection fraction, indexed RV end-diastolic and end-systolic volumes, and VVCR (all $P < 0.001$).
Table 11. RV correlations with measures of MPA stiffness

<table>
<thead>
<tr>
<th></th>
<th>PWV (m/s)</th>
<th>RAC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>-0.120 ± 0.018, 0.657</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>VVCR*</td>
<td>3.05 ± 0.45, 0.662</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CI</td>
<td>0.038 ± 0.159, 0.202</td>
<td>0.8114</td>
</tr>
<tr>
<td>EDVi</td>
<td>0.028 ± 0.005, 0.595</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ESVi</td>
<td>0.037 ± 0.005, 0.691</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SVi</td>
<td>-0.16 ± 0.018, 0.226</td>
<td>0.3874</td>
</tr>
</tbody>
</table>

Data are reported as beta coefficients ± SEM, R-Value and P values. PWV = pulse wave velocity, RAC = relative area change, EDVi = end-diastolic volume index, ESVi = end-systolic volume index, SVi = stroke volume index, VVCR = ventricular-vascular coupling ratio, EF = ejection fraction, CI = cardiac index.* Parameter was log transformed. All correlations are adjusted for age, sex, and BSA.

**Longitudinal MPA Stiffness and WSS Analysis**

The bivariate random coefficient model was applied to estimate and correlate random slopes of MPA stiffness indices with WSS$_{\text{max}}$ to investigate the mechanism of shear mediated vascular remodeling (Figure 5). The average time between two CMR acquisitions was 3.5 years (range: 0.6 to 6 years). Trends in WSS$_{\text{max}}$ were significantly correlated with RAC ($R$ value = 0.679, $P = 0.0007$). However, the relationship in trends between the WSS$_{\text{max}}$ and PWV failed to reveal significant relationship ($R$ value = -0.359, $P = 0.1101$).

![Figure 24](image)

**Figure 24.** A) Random slope analysis depicting the trend between PWV and WSS$_{\text{max}}$ failed to reveal significant relationship between two metrics. B) Contrary, same analysis between the RAC and WSS$_{\text{max}}$ revealed significant positive trend.
Predictive Analysis

With respect to severe clinical outcomes, the average follow-up time was 2.2 years (range 0.2 to 7.4 years) in which three patients died, one underwent lung transplantation, six were initiated on intravenous or subcutaneous prostanoid therapy, two received clinically indicated atrial septostomy, and one underwent a Pott’s shunt. For the moderate clinical outcomes, the average follow-up time was 2.1 years (range 0.2 to 8.1 years) during which 20 patients had worsening of WHO-FC, 4 patients experienced syncopal event during clinical visits, three patients had to be hospitalized due to PAH related causes, and one patient experienced hemoptysis. The univariate proportional hazard analysis is summarized in Table 12. From all considered MPA and RV specific metrics, only the PWV was shown to have a prognostic potential toward moderate clinical outcomes. For each unit increase in PWV, there was approximately a 3.2-fold increase in having a moderate clinical event. The corresponding Kaplan-Meyer plot is depicted in Figure 25 with the receiver-operating cut-off value of 2.3 m/s. None of the considered metrics showed potential toward predicting severe clinical event.

| Table 12. Univariate Proportional Hazard Analysis |
|-----------------------------------------------|-----------------|-----------------|
| Moderate Outcomes (n = 28) | Severe Outcomes (n = 13) |
| **HR (95% CI)** | **P value** | **HR (95% CI)** | **P value** |
| PWV | 3.18 (1.44 – 7.73) | **0.0063** | 3.15 (0.98 - 10.06) | 0.0541 |
| RAC | 0.75 (0.34 - 1.62) | 0.4672 | 0.69 (0.18 - 2.56) | 0.5816 |
| WSS<sub>max</sub> | 0.72 (0.32 - 1.64) | 0.4374 | 0.17 (0.02 - 1.32) | 0.0906 |
| VVCR | 1.30 (0.52 - 3.26) | 0.5656 | 2.70 (0.59 - 12.39) | 0.2003 |
| EF | 0.54 (0.24 - 1.19) | 0.1298 | 0.45 (0.15 - 1.44) | 0.1834 |
| EDVi | 1.34 (0.62 - 2.88) | 0.4466 | 2.25 (0.67 - 7.51) | 0.1844 |
| ESVi | 1.23 (0.57 - 2.64) | 0.5876 | 2.41 (0.65 - 8.96) | 0.1873 |
| SVi | 0.75 (0.35 - 1.63) | 0.4748 | 0.31 (0.09 - 1.05) | 0.0612 |

Data reported as Cox proportionate hazard ratios with corresponding 95% confidence intervals. *P < 0.05. n = number of adverse events in each category. PWV = pulse wave velocity, RAC = relative area change, WSS<sub>max</sub> = maximum systolic wall shear stress, EDVi = end-diastolic volume index, ESVi = end-systolic volume index, SVi = stroke volume index, VVCR = ventricular-vascular coupling ratio, EF = ejection fraction
Discussion

In this study, we have shown that non-invasive markers of proximal pulmonary vascular stiffness are significantly elevated in the pediatric PAH population, that stiffness metrics representing additional RV afterload are related to RV size and function, and that progression in stiffness could be associated with changes in flow hemodynamic forces acting on the vessel wall. Furthermore, we found that stiffness measured in the MPA can be predictive of moderate clinical events. While the stiffness of proximal pulmonary conduit vessels has been described in studies considering heterogeneous PAH populations$^{62,64}$, this is the first study to demonstrate the potential prognostic utility of non-invasive and comprehensive evaluations of MPA stiffness in children with PAH.

MPA stiffness in pediatric PAH

In our previous work, we have described significantly reduced WSS metrics and RAC in smaller pediatric PAH populations$^{42,105}$. However, RAC can be considered a simplified metric of MPA stiffness independent of pulse pressure, whereas PWV is considered gold-standard non-invasive index of vascular stiffness$^{69}$. PWV is specifically applicable for vessel stiffness analysis given that considerable wall remodeling may occur prior to alterations in vessel geometry.
Additionally, PWV was investigated in this study using flow-area method which enables region and plane specific analysis which should be considered in this patient population given the heterogeneity of pulmonary arterial wall composition and backward wave reflections coming from the proximal pulmonary arterial branches\textsuperscript{33,109}. In this study, we have observed nearly two-fold increase in PWV measured at the MPA, similar to a previously reported adult study\textsuperscript{55}. Additional studies in the adult literature have reported dramatically decreased RAC along with the MPA compliance and distensibility\textsuperscript{82,110}. Other studies have suggested that non-invasive detection of MPA stiffness is an early sign of worsening pulmonary vascular disease correlating with clinically prognostic outcomes, and that stiffness can significantly increase with response to exercise induced stress\textsuperscript{61,62,111,112}. Unfortunately, serial follow-up of pediatric PAH patients by combined catheterization and advanced imaging techniques is challenging due to the invasiveness of cardiac catheterization. Additionally, comprehensive evaluation of pulmonary vascular stiffness using impedance frequency domain analysis along with more simple pulse pressure based metrics (i.e. compliance and distensibility) require catheter derived pressure waveform and are limited for clinical evaluation of pulmonary circulation, especially in children \textsuperscript{54,113}. Indeed, majority of consensus recommendations suggest non-invasive clinical follow-up evaluation by means of echocardiography or CMR\textsuperscript{22}. The promising route toward more comprehensive non-invasive characterization of PAH is real-time CMR allowing for high temporal resolution imaging, but this technique is currently applied only in a limited number of institutions due to high post-processing time\textsuperscript{55,73,114}.
MPA stiffness and RV afterload

The significant contribution of increased proximal pulmonary vascular stiffness to RV afterload has been demonstrated in both adult and pediatric PAH populations\textsuperscript{15,19,33,76}. In this study, we have shown significant correlations between non-invasive markers of MPA stiffness with volumetric and functional RV indices. Non-invasive evaluation of RV remodeling as a response to elevated afterload has been previously investigated by means of a simplified version of the VVCR (mathematical transform of RV ejection fraction), demonstrating potential for describing disease severity and prediction of clinical outcomes\textsuperscript{16,17,115}. Strong association between PWV and RAC with VVCR in our study suggests that critical contribution of proximal stiffness to RV-pulmonary artery axis function can be measured non-invasively in pediatric PAH population and further supports ongoing efforts for finding therapeutic targeting for proximal vascular stiffness\textsuperscript{33,116}.

Shear Hemodynamics and Stiffness

Flow mediated remodeling of pulmonary and systemic vasculature is already recognized phenomenon applicable to broad range of vasculopathies\textsuperscript{26,72}. The mechanotransduction driven endothelial and extra-cellular matrix remodeling in PAH has been described in several in-vitro and animal studies\textsuperscript{8,33,76,86,87,96}. Similar to systemic circulation, shear mediated response in pulmonary circulation has a spatially heterogeneous response i.e. endothelial cells in the pulmonary artery will have different responsiveness to shear abnormalities than those at the level of pulmonary arterioles\textsuperscript{29,32,117}. Furthermore, endothelial response to WSS variations is magnitude and direction specific, with low and oscillatory shear mediating endothelial cell proliferation and activation of the local inflammatory response\textsuperscript{26,27,118}. Indeed, in the presented
study we have observed dramatically reduced WSS\textsubscript{max} and WSS\textsubscript{TA} along with elevated OSI in the presence of increased MPA stiffness. In the presence of such a condition, the extra-cellular matrix of the MPA has been shown in in-vitro studies to remodel toward collagen dominant and thus, stiffer, character\textsuperscript{86,119}. Importantly, we observed that changes throughout the disease progression in the WSS\textsubscript{max} correspond to the changes in RAC representing a purely geometric assessment of vascular deformation. Interestingly, the PWV has not shown a significant correlation but was indicative of the corresponding trend. While the major theoretical determinants of WSS, vessel size, was severely enlarged in PAH, one must carry in mind that theoretical WSS as calculated by Haagen-Poiseuille law relationship assumes ideal pipe-flow condition and does not account for pulsatile flow and three-dimensional secondary flow structures (vortices and helices), which are often associated with PAH\textsuperscript{40}. Further and more comprehensive studies using four-dimensional flow CMR (4D-Flow CMR) enabling broad three-dimensional WSS analysis will be required to study the relationship between WSS and proximal pulmonary stiffness in pediatric patients. A previous adult study has successfully described reduced WSS assessed by 4D-Flow CMR in the MPA and correlated shear metrics to invasive hemodynamic indices\textsuperscript{40}. Other qualitative and quantitative flow hemodynamic metrics of flow disturbances affecting WSS have been correlated with the severity of PAH\textsuperscript{104}.

*Prognostic value of the MPA stiffness*

Several catheterization-derived markers, standard clinical indices, and non-invasive RV echocardiographic and CMR derived specific metrics have been previously shown to be strongly associated with clinically important outcomes in children with PAH. Specifically, MPA stiffness derived indices, reflective of pulsatile load derived by catheterization, have been shown to have
good prognostic potential to predict PAH related morbidity and mortality\textsuperscript{120,121}. However none to date have evaluated the prognostic ability of non-invasive imaging stiffness markers in pediatric PAH population\textsuperscript{73,122}. In this study, we considered two different sets of composite outcomes and found that MPA stiffness represented by PWV can be predictive of moderate clinical events. Interestingly, none of the other RV metrics were shown to be predictive of moderate or severe clinical outcomes. We speculate that this is mainly due to smaller number of patients considered for prognostic analysis with respect to previously described large studies and the nature of composite outcomes. Moledina et al has shown significant prognostic potential of RV and left ventricular volumetric indexed measures toward freedom from death or transplantation\textsuperscript{73}. Measuring meaningful clinical outcomes in both adult and pediatric populations remains challenging; while more evaluation is needed to find suitable clinical endpoints for this population, composite clinical outcomes made up of less dramatic clinical events should be a priority. Previous work has shown the predictive ability of RAC toward mortality in broad PAH adult populations\textsuperscript{62}. Furthermore, invasive stiffness metrics sampled from young children with suspected pulmonary vascular disease has been shown to have prognostic value for PAH severity and the development of PAH in adulthood\textsuperscript{57}. In this work, we showed that non-invasively derived PWV, a surrogate for MPA stiffness can predict adverse clinical outcomes.

\textit{Limitations}

Retrospective analysis of CMR in children with PAH is limiting as there was some heterogeneity in CMR acquisition. The variability in different MRI systems may indeed introduce inter-system variability, however the previous studies have reported limited effect of different field strength on hemodynamic measurements\textsuperscript{48}. The sample size of our PAH cohort
limited both baseline and prognostic analysis. This is mainly due to fact that young children (< 7 years of age) require sedation for CMR evaluation per institutional protocol. Additionally, severe outcomes are infrequent in the pediatric PAH population lacking a consensus definition of moderate and severe outcomes\textsuperscript{22}. Retrospective analysis also limits the ability to coordinate CMR with invasive catheterization and therefore clinical decisions made between catheterization and CMR may contribute to altered outcomes. Lastly, our PAH patient population might differ from other centers due to regional factors associated with high altitude. These limitations have been postulated previously as unavoidable in majority of pediatric CMR studies\textsuperscript{106}.

A technical limitation is through plane motion of the pulmonary trunk, which may have introduced an error to both PWV (dQ/dA method) and RAC analysis. Furthermore, the PWV measured by flow wave propagation within the proximal pulmonary conduit in children is limited by the spatiotemporal resolution and is more applicable to the aortic stiffness analysis. Here, we attempted to mitigate all potential errors with backward wave reflections in pulmonary vessels by secluding PWV analysis only to the specific portion of the flow upstroke waveform without evidence of a wave reflection.

Conclusion

Children with PAH have increased pulmonary vascular stiffness as assessed by non-invasive CMR evaluation regardless of PAH etiology. Metrics of MPA stiffness also correlate with RV volumetric and functional indices implying an important contribution to the RV afterload. MPA stiffness is associated with reduced hemodynamic WSS, and changes in both metrics followed a consistent pattern on follow up CMR. Importantly, we found that PWV measured in the MPA can be predictive of moderate clinical outcomes and therefore may be
applied toward CMR follow up evaluation of pediatric PAH patients. The presence of proximal pulmonary vascular stiffness in PAH then should be further considered as therapeutic target in pediatric population.
CHAPTER V

CONCLUSION

Vascular Remodeling-Flow Interactions in Pediatric Pulmonary Hypertension

In summary, performed studies indicated that: 1) proximal pulmonary arteries in children with PAH show clear evidence of elevated stiffness which is prognostic of clinical and functional worsening, 2) severity of the pulmonary arterial stiffness is associated with reduced hemodynamic WSS, and 3) the longitudinal changes in WSS and geometry relates strain based stiffness throughout the disease progression are interrelated. Pulmonary arterial remodeling mediated by flow hemodynamic forces is a well-recognized feature of PAH, which has been predominantly investigated at the level of resistant arterioles and microvascular endothelium. The presented series of studies indicates that shear mediated vascular stiffening may have further implications in remodeling of large proximal pulmonary arteries. Yet, it should be recognized that it remains impossible to provide direct causality between hemodynamic shear stress and vascular stiffness, and to determine whether the changes in flow and shear are early contributors or solely augmenting factors of proximal pulmonary arterial remodeling in pediatric PAH, mainly because all patients considered in the discussed studies were in progressed stage of the disease. Further clinical or animal imaging studies associated with the immuno-histochemistry assessment of proximal pulmonary arterial wall tissue are warranted to provide further insights into regional WSS abnormalities and vascular remodeling.

The defective endothelial response to volume overload, shear abnormalities, and overall flow disruptions appears to be primarily modulated by plasma endothelin cell adhesion molecule 1 (PECAM-1). Previous studies suggested that pathological response to reduced and oscillatory WSS is represented by formation of leaky and sticky endothelial cells, increased
presence of reactive oxygen species, increased rate of inflammatory cell infiltration, higher rate of matrix-metalloprotein proteases synthesis by macrophages, and smooth muscle cell proliferation and infiltration\textsuperscript{26,118,123}. However, as suggested by study by Szulcek et al, vascular remodeling can be decelerated or reversed by restoration of PECAM-1 signaling despite of continual WSS abnormalities. Consequently, novel therapeutic strategies targeting distal vascular remodeling might be further beneficial for the restoration of proximal pulmonary arterial compliance as well.

**Benefits of Non-invasive MRI Imaging**

It should be highlighted that all flow hemodynamic phenomenon described in previous chapters have been performed using non-invasive phase-contrast MRI. PC-MRI can provide sufficient spatiotemporal analysis to perform comprehensive flow, shear, and wave-intensity studies from a single acquisition. The non-invasive nature of PC-MRI and short acquisition time is ideal for pediatric flow hemodynamic evaluation and may offer a novel route of absolute flow and resistance / stiffness assessment particularly when combined with cardiac catheterization. Additional appeal of PC-MRI for measuring flow through proximal pulmonary conduit arteries in children lies in the ability of internal validation of flow calculation through the main pulmonary artery and its primary branches with good reproducibility\textsuperscript{43,124}. Unfortunately, comprehensive MRI studies in younger children and infants typically mandate the use of general anesthesia, thereby introducing a level of invasiveness and potential cardiovascular hemodynamic changes associated with anesthesia. Furthermore, severely decompensated children might have problem with a standard breath-held acquisitions.
Overall, non-invasive flow hemodynamic evaluation via PC-MRI is feasible in children with PAH and might become a routine component of clinical evaluation in pediatric PAH providing comprehensive evaluation of pulmonary arterial stiffness, flow hemodynamic status, and clinically meaningful biomarkers predictive of functional worsening.

**Future Directions**

Future longitudinal prospective studies utilizing comprehensive cardiac and PC-MRI evaluation should be employed to fully investigate flow – stiffness relationship, ventricular-vascular coupling, and even flow – tissue composition associations. Recent study by Szulcek et al described the role of defective PECAM-1 signaling in response to high shear stress presented in microvascular endothelial cells cultured from resected lung tissue of patients with end-stage PAH, and found that PECAM-1 signaling can be restored by means of pan-caspase inhibition resulting in disease amelioration\(^{32}\). Whether abnormally low WSS can induce similar response in proximal large pulmonary arteries is yet to be determined. The promising modality capable of comprehensive flow hemodynamic mapping is 4D-Flow MRI which can further provide comprehensive WSS analysis through-out the entire proximal pulmonary arteries, calculation of kinetic energy loss by non-laminar flow, and intra-cardiac flow evaluation sensitive to ventricular relaxation properties and overall diastolic function.

Next studies will focus on utilization of 4D-Flow MRI in combination with invasive catheter-based hemodynamics. Overarching goal of this study will be to show the association of flow hemodynamic shear forces measured by 4D-Flow MRI with harvested pulmonary arterial endothelial cells derived during a same-day right heart catheterization in children with PAH. Cross-correlation of flow hemodynamics with the locally corresponding tissue characteristics has
been previously possible only in the systemic circulation, in patients with bicuspid aortic valve undergoing prophylactic ascending aortic resection due to excessive dilation. Unfortunately, similar studies in the context of PAH would possible only in patients undergoing lung transplantation or in animal models.

Recently, characterization of pulmonary arterial endothelial cells has been possible in PAH patients using cells harvested by means of right heart catheterization. Specifically, Benza et al showed that endothelial cells adhered to the inflated elastic balloon attached to standard Swan-Ganz catheter can be isolated, expended, and characterized using specific incubation technique designed for pulmonary arterial endothelial cells isolation. Future studies will be performing the similar type of analysis in pediatric PAH patients in combination with the same-day 4D-Flow MRI in order to establish for the first time relationship between flow hemodynamic forces and endothelial cell function in the pulmonary arterial system.

Additionally, evaluation of flow prior to catheterization or in combination using hybrid MRI catheterization procedures may reduce radiation dose during catheterization and time of anesthesia / sedation. Lastly, the MRI-based vasoreactivity testing may offer novel set of parameters in addition to standard catheterization and provide more robust volumetric and flow information crucial for the determination of ventricular–vascular coupling and proximal pulmonary arterial remodeling.
REFERENCES


