

**THE IN VIVO COMPARISON OF INVASIVE AND NON-
INVASIVE ASSESSMENTS OF PULMONARY VESSEL
HAEMODYNAMICS AND VASOREACTIVITY IN PATIENTS
WITH KNOWN OR SUSPECTED PULMONARY ARTERIAL
HYPERTENSION: A CARDIAC MAGNETIC RESONANCE**

IMAGING STUDY

A thesis submitted by:

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For the degree of:

Doctor of Philosophy

**Discipline of Medicine
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THESIS RELATED PUBLICATIONS

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2. Baillie TJ, Sidharta S, Steele PM, Worthley SG, Willoughby S, Teo K, Sanders P, Nicholls SJ, Worthley MI. The predictive capabilities of a novel cardiovascular magnetic resonance derived marker of cardiopulmonary reserve on established prognostic surrogate markers in patients with pulmonary vascular disease: results of a longitudinal pilot study. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2017;19:3.

Submitted Manuscript

1. Baillie TJ, Sidharta S, Steele PM, Worthley SG, Willoughby S, Teo K, Sanders P, Nicholls SJ, Worthley MI. The relationship between coronary flow reserve, myocardial blood supply:demand balance, disease severity, and cardiopulmonary reserve in patients with pulmonary vascular disease. Under review at: *International Journal of Cardiology*.

Published Abstracts

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without pulmonary arterial hypertension. *Heart, Lung and Circulation*, 2015, 24:S318.

- 2.** Baillie TJ, Sidharta S, Steele PM, Worthley SG, Willoughby S, Teo K, Sanders P, Nicholls SJ, Worthley MI. A novel non-invasive pulmonary vasoreactivity protocol utilising cardiac magnetic resonance imaging in patients with known or suspected pulmonary arterial hypertension (PAH). *Heart, Lung and Circulation*, 2015, 24:S318.

ABBREVIATIONS

6MWD: 6-minute walk distance

AL: afterload

BSA: body surface area

CI: cardiac index

CMR: cardiac magnetic resonance imaging

CO: cardiac output

CSF: coronary sinus flow

CSFR: coronary sinus flow reserve

DPTI: diastolic pressure-time index

iPAH: idiopathic pulmonary arterial hypertension

IV: intravenous

LA: left atrium

LV: left ventricle

meanPAvel: mean pulmonary arterial blood flow velocity

mPAP: mean pulmonary arterial pressure

mPAWP: mean pulmonary arterial wedge pressure

NTpBNP: N-terminal pro-brain natriuretic peptide

PA: pulmonary artery

PAH: pulmonary arterial hypertension

PI: pressure index

PVD: pulmonary vascular disease

PVR: pulmonary vascular resistance

RA: right atrium

RAP: right atrial pressure

RHC: right heart catheter

RHC: right heart catheter

RV: right ventricle

RVEDVI: indexed right ventricle end diastolic volume

RVEF: right ventricular ejection fraction

RVESVI: right ventricular end systolic volume indexed

SPTI: systolic pressure-time index

SSFP: steady-state free precession

SVI: indexed stroke volume

TTI: tension-time index

WU: Wood units

THESIS DECLARATION

NAME: Timothy J.G. BAILLIE

DEGREE: Doctor of Philosophy

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signature:

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CHAPTER 1

INTRODUCTION, THESIS OVERVIEW, AND BACKGROUND

STATEMENT OF AUTHORSHIP

Title of Paper	INTRODUCTION, THESIS OVERVIEW, AND BACKGROUND
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Principal Author

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Overall percentage (%)	95		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	27/6/18

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Matthew WORTHLEY		
Contribution to the Paper	Correction and critical review.		
Signature		Date	25/6/18

1.1 INTRODUCTION AND THESIS OVERVIEW

Pulmonary hypertension (PH), a pathophysiologic condition defined by a mean pulmonary artery pressure (mPAP) of $>25\text{mmHg}$ measured at rest by right heart catheter (RHC), can complicate a range of respiratory, cardiac, and systemic diseases (Figure 1)[1]. Pulmonary arterial hypertension (PAH; Group 1 PH) is characterized by a pathological rise in the resistance of the pulmonary circulation due to obliterative plexogenic lesions at the proximal microcirculatory level. This distinct pulmonary vascular pathophenotype is diagnosed by the presence of pre-capillary PH (mean pulmonary arterial wedge pressure [mPAWP] $<15\text{mmHg}$ and pulmonary vascular resistance [PVR] >3 Wood Units) and the absence of significant lung disease (Group 3 PH) or chronic thromboembolic disease (Group 4 PH). PAH is considered a rare disease with an annual incidence of approximately 2.4 – 10 cases per million per year, with about half being sporadic (idiopathic), heritable, or drug-induced, while systemic sclerosis is the most common cause of associated PAH (APAH) [2-4].

Occlusive pulmonary vascular lesions are caused by excessive vasoconstriction, arterial wall remodelling, and in situ thrombosis propagated by pathological interactions between growth factors and their receptors, neurohormones, inflammatory cytokines, endothelial cell dysfunction, and dysregulated cell growth and division. Loss of effective pulmonary vascular cross-sectional area increases pulmonary vascular resistance (PVR) and total RV afterload, placing undue stress on the RV. Therefore, while first and foremost a pulmonary ‘vasculopathy’, morbidity and mortality is closely linked to the functional capacity of the

stressed RV. Over the past two decades, advances in disease understanding and an enriched therapeutic armamentarium have improved clinical outcomes but PAH remains a progressive, incurable disease with an unacceptably high short-term mortality (3-year median survival rates of 58% - 72%), predominantly due to RV failure [5, 6].

Given the absence of curative treatments, there is a pressing need to identify means by which to improve clinical outcomes using current therapies. The goal of the present body of work is to investigate whether a standardised, non-invasive pulmonary vasoreactive challenge utilizing cardiac magnetic resonance imaging (CMR) and intravenous adenosine infusion can provide novel insight into the status of the pulmonary vascular bed, the RV, and their interaction (i.e. the cardiopulmonary unit), and to determine whether this may afford a means to detect early pulmonary vascular disease (i.e. before measureable resting pulmonary haemodynamic abnormalities arise) and objectively monitor disease progression over time. The ability to detect early PVD would represent a paradigm shift in how PAH is defined and managed, while a standardized, unified method to assess disease progression and treatment response would facilitate timely clinical decision-making (e.g. institution of advanced medical therapies such as parenteral prostanoids, or referral for lung transplantation).

The present Chapter details physiologic principles underlying the protocol design including a discussion regarding the paradigm of early disease detection, current screening practices and their limitations, exercise pulmonary haemodynamic assessment of PVD, prognostic importance of acute vasoreactivity testing, the concept of pulmonary flow reserve (PFR), and limitations of current risk assessment in established PAH. The importance of functional adaptation of the RV is addressed, with emphasis on the relationships between RV coronary perfusion, RV remodelling and performance, and cardiopulmonary reserve.

Since this body of work is centred around a novel, non-invasive pulmonary vasoreactive protocol, a separate (brief) methodology section is provided in Chapter 2. Here, cardiac magnetic resonance imaging sequences, adenosine infusion dosing, and the invasive vasoreactivity protocol are outlined and justified.

Chapters 3-5 comprise the results section of the thesis. Chapter 3 details findings from the baseline assessment of the study cohorts including safety and tolerability of the protocol, validity of CMR findings using invasive haemodynamics during vasoreactivity as the comparator, and the application of findings towards detection of early PVD. Chapter 4 details relationships between CMR-derived parameters measured at rest and during adenosine-stress with validated invasive and non-invasive prognostic markers used in established PAH. This chapter covers findings from the initial assessment, as well as longitudinally over a 6-month period.

Chapter 5 focuses on the relationships between coronary micro- and macro-vascular status measured by CMR-derived coronary sinus flow reserve and RHC-derived myocardial supply:demand indexes, PVD burden (RV afterload), RV remodelling and performance, and cardiopulmonary reserve. A brief conclusion is included in Chapter 6, along with a forward-looking statement.

1. Pulmonary arterial hypertension
<ul style="list-style-type: none"> 1.1 Idiopathic 1.2 Heritable <ul style="list-style-type: none"> 1.2.1 BMPR2 mutation 1.2.2 Other mutations 1.3 Drugs and toxins induced 1.4 Associated with: <ul style="list-style-type: none"> 1.4.1 Connective tissue disease 1.4.2 Human immunodeficiency virus (HIV) infection 1.4.3 Portal hypertension 1.4.4 Congenital heart disease (Table 6) 1.4.5 Schistosomiasis
1'. Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
<ul style="list-style-type: none"> 1'.1 Idiopathic 1'.2 Heritable <ul style="list-style-type: none"> 1'.2.1 EIF2AK4 mutation 1'.2.2 Other mutations 1'.3 Drugs, toxins and radiation induced 1'.4 Associated with: <ul style="list-style-type: none"> 1'.4.1 Connective tissue disease 1'.4.2 HIV infection
1". Persistent pulmonary hypertension of the newborn
2. Pulmonary hypertension due to left heart disease
<ul style="list-style-type: none"> 2.1 Left ventricular systolic dysfunction 2.2 Left ventricular diastolic dysfunction 2.3 Valvular disease 2.4 Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies 2.5 Congenital/acquired pulmonary veins stenosis
3. Pulmonary hypertension due to lung diseases and/or hypoxia
<ul style="list-style-type: none"> 3.1 Chronic obstructive pulmonary disease 3.2 Interstitial lung disease 3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern 3.4 Sleep-disordered breathing 3.5 Alveolar hypoventilation disorders 3.6 Chronic exposure to high altitude 3.7 Developmental lung diseases (Web Table III)
4. Chronic thromboembolic pulmonary hypertension and other pulmonary artery obstructions
<ul style="list-style-type: none"> 4.1 Chronic thromboembolic pulmonary hypertension 4.2 Other pulmonary artery obstructions <ul style="list-style-type: none"> 4.2.1 Angiosarcoma 4.2.2 Other intravascular tumors 4.2.3 Arteritis 4.2.4 Congenital pulmonary arteries stenoses 4.2.5 Parasites (hydatidosis)
5. Pulmonary hypertension with unclear and/or multifactorial mechanisms
<ul style="list-style-type: none"> 5.1 Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy 5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangiomyomatosis, neurofibromatosis 5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders 5.4 Others: pulmonary tumoral thrombotic microangiopathy, fibrosing mediastinitis, chronic renal failure (with/without dialysis), segmental pulmonary hypertension

Figure 1: Clinical classification of pulmonary hypertension.

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1.2 BACKGROUND

1.2.1 EARLY DETECTION OF PAH

The healthy pulmonary vascular bed is a low pressure, low resistance circuit with high capacitance and large microcirculatory reserve which, even during exercise, can accommodate high cardiac output (CO) with minimal impact on pressure [8, 9]. Obstruction of the vascular bed in the context of PVD can progress unnoticed until the recruitable circulatory reserve is overwhelmed. Following the loss of approximately 50% of the microcirculation, symptoms typically develop (usually exertional dyspnoea) and measureable changes in resting pulmonary haemodynamics arise (Figure 2) [10, 11]. This, combined with the often-lengthy delay between symptom onset PAH diagnosis [12], ensures that the vasculopathic burden is uniformly high at diagnosis, even if haemodynamic perturbations are 'mild'.

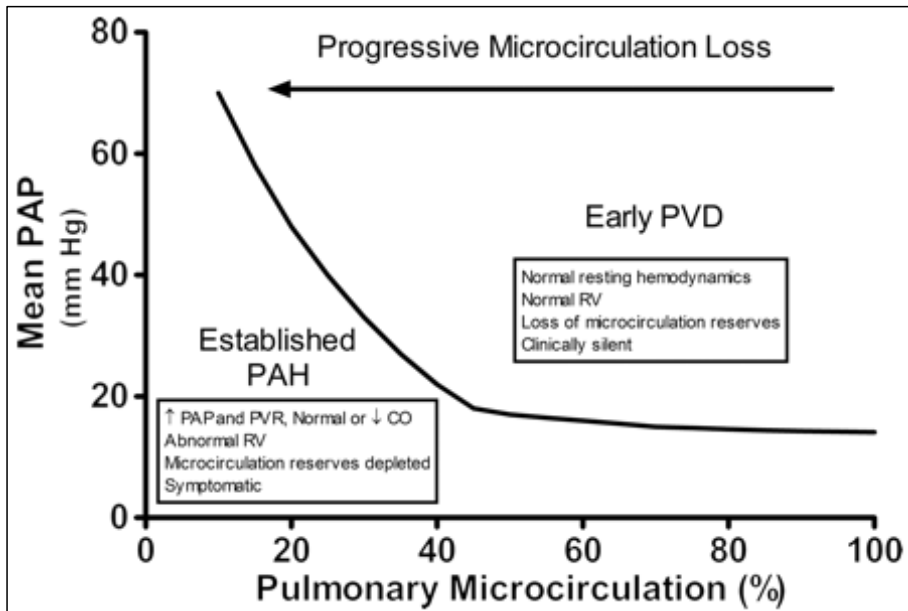


Figure 2: Schema of PAH progression (PVD = pulmonary vascular disease).

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While the duration between onset of PVD, development of clinical symptoms, and presence of diagnostic haemodynamic abnormalities is unclear, data supports the hypothesis that earlier intervention with effective medical therapies improves clinical outcomes in those with PAH. This is perhaps a logical concept given targeted therapies (endothelin receptor antagonists, phosphodiesterase inhibitors, soluble guanylate cyclase activators, and prostacyclin analogues) exert antiproliferative and antimitogenic effects in addition to potent vasodilation, and should, therefore, ameliorate the natural history of the disease [13]. This is supported by clinical trial data showing PAH therapies delay clinical worsening and improve long term outcomes, even in ‘mild’ disease (i.e. NYHA Class II) [14-16]. Data for the recent AMBITION trial showed improved survival with initial combination therapy (ambrisentan and tadalafil) over monotherapy (tadalafil) [17]. In APAH, patients with

clinically advanced disease (functional class III/IV) at diagnosis had significantly worse prognoses than those with mild symptoms [18-20] [21]. Finally, follow up to the ItinerAir study showed scleroderma-associated PAH identified through active screening had better survival when compared to a parallel group identified through standard clinical practice [22], and while this study was limited by lead-in bias and small numbers, it provides conceptual support for early intervention.

Identifying disease early with a view to intervening and mitigating progression requires a screening process which, ideally, should be non-invasive, safe, ~~effective~~, ~~cheap~~ cost effective, observer independent, standardised, sensitive and specific, and widely available. Any screening strategy must target certain populations with a sufficiently high absolute risk of the target disease to avoid high rates of false positive and negative results. Current international guidelines recommend screening for PAH in patients with systemic sclerosis (SSc), bone morphogenic protein receptor type 2 (BMP2) mutation carriers or their first-degree relatives (heritable PAH), and those with portal hypertension referred for liver transplantation [7]. Prevalence of PAH in the general population is estimated at 15-60 cases per million (up to 0.006%) [2] compared with 8-10% in the SSc population, 20% in BMP2 mutation carriers, and 0.5-10% in portal hypertension, and absolute lifetime risk for PAH is sufficiently high in these groups to warrant screening (10-20%) [2, 3, 23-25].

Since there is no validated method to detect early PVD (i.e. before haemodynamic abnormalities arise), established screening tests target the non-invasive identification of PH and its sequelae (e.g. right heart strain). Transthoracic Doppler echocardiography (TTE) is the cornerstone of this approach, using the simplified Bernoulli equation to estimate the pulmonary arterial systolic pressure (PASP) from the tricuspid regurgitant jet velocity (TRV)

and estimated right atrial pressure (RAP; estimated by assessing inferior vena cava size and collapsibility with inspiration). While widely available, cheap, and safe, it is limited by sonographer experience, body habitus, image quality, and the dependence on an adequate TR Doppler profile, which may be unobtainable in 10-20% of patients being assessed for possible PH [26]. There are also concerns regarding the precision of TTE-estimated pulmonary pressures [27, 28], particularly at lower TR jet velocities [29]. The performance of resting TTE to identify PH is also highly dependent upon the chosen threshold for the TRV. A prospective study in SSc patients found PH could be reasonably detected using a TRV of >3 meters/second (m/s) irrespective of symptoms, or 2.5-3 m/s in conjunction with unexplained dyspnoea, although false positives (and excess RHCs) were high at about 45% [23]. Current European guidelines suggest a high echocardiographic probability for PH when the TRV is >3.4 m/s or 2.9-2.4 m/s with other signs of PH (e.g. dilated RV, flattened interventricular septum, enlarged RA, high estimated RAP); intermediate with TRV 2.9-3.4 m/s or ≤2.8 m/s with other PH signs; and low when TRV ≤2.8 m/s without other PH signs (Figure 3).

Peak tricuspid regurgitation velocity (m/s)	Presence of other echo 'PH signs' ^a	Echocardiographic probability of pulmonary hypertension
≤2.8 or not measurable	No	Low
≤2.8 or not measurable	Yes	Intermediate
2.9-3.4	No	
2.9-3.4	Yes	High
>3.4	Not required	

Figure 3: Echocardiographic probability of PH.

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Other screening tools include lung function testing (principally assessing the diffusing capacity of the lungs for carbon monoxide [DLCO] which, if reduced, may suggest a low pulmonary capillary blood volume due to PVD) and serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), which rise with ventricular volume and pressure overload [30]. An increased risk of PAH in SSc patients with a DLCO \leq 60%, an elevated NT-proBNP alone, or an elevated NT-proBNP in conjunction with a low DLCO has been demonstrated, although both tests lack the necessary sensitivity and specificity for PH to be used in isolation [23] [31-33]. Screening algorithms employing a combination of tools have been studied with variable reported success [34-38], although all studies were limited by an inability to determine the false negative rate as RHC was not performed in control groups. Furthermore, entry criteria often favoured more symptomatic patients with higher pre-test suspicion for PAH.

The cross-sectional, multi-centre DETECT trial was the first to address the false negative rate of a screening algorithm [39]. Patients with SSc for $>$ 3 years and DLCO $<$ 60% underwent a range of non-invasive investigations including clinical assessment, serum biomarkers, electrocardiography, and resting TTE before proceeding to RHC. PAH was found in 87 of the 466 patients (19%) and was mild in the majority (64% WHO functional I/II). The best discriminatory variables for PAH detection were determined and a two-step algorithm was proposed: step 1 determined referral for resting TTE based on six simple clinical variables, and step 2 determined referral for RHC based on the step 1 risk score and subsequent TTE findings. Internal validation showed that 62% of patients would be referred for RHC, with a sensitivity of 96% and specificity of 48% for PAH detection (false negative rate of 4%). This outperformed European guideline recommendations of annual echocardiography in symptomatic SSc patients, with a higher sensitivity and similar specificity [40], although it

remains unclear how to proceed in patients with a DLCO >60% and the algorithm lacks validation in larger cohorts.

1.2.2 EARLY DETECTION OF PVD

Exercise Pulmonary Haemodynamics

As noted, established screening programs target non-invasive detection of PH or its sequelae. Presently, no validated method exists to reliably identify PVD prior to overt PAH. A rational method to detect early stages of disease, with a view to intervening before adverse consequences arise, is to assess for an impaired 'reserve' by stressing the relevant system and detecting abnormal physiologic responses. Since symptoms with exertion precede symptoms at rest and an impaired pulmonary vascular reserve precedes PAH, pathophysiologic rationale for exercise haemodynamic testing of patients with suspected PAH/early PVD is impeccable, but is limited by several factors. Firstly, pulmonary haemodynamic changes during exercise are complex and less well understood than the systemic circulation, and the bounds of 'normal' physiology are unclear. Exercise-induced PH (eiPH) was originally defined as a mPAP>30mmHg during exercise [41] until it was observed that this threshold was not uncommon in 'normal' individuals and was often exceeded in conditioned athletes [42-44]. This is explained by the mPAP being dependent on PVR, left atrial pressure (LAP), and cardiac output (CO), so that significant CO augmentation (e.g. athletes) or an abnormal PVR (e.g. PVD) response can similarly elevate mPAP during exercise, even when LAP response is normal.

A more discerning method to detect a compromised pulmonary circulation is to analyse the slope reflecting the multi-point relationship between flow (CO) and pressure (mPAP)

acquired during incremental exercise [10, 45]. In this context, a steeper than normal slope would be expected (i.e. pressure rising rapidly in relation to flow, assuming a normal LAP response) due to a blunted capacity to recruit the pulmonary microcirculation [42]. While Castelain *et al* have shown a decrease in this slope after six weeks of IV epoprostenol therapy in treatment-naïve patients with severe PAH (Figure 4) - despite no change in resting haemodynamics but a rise in exercise capacity [45] - its application to the detection of early PVD is hampered by an incomplete understanding of a normal slope [43] and a dependence on invasive measurements.

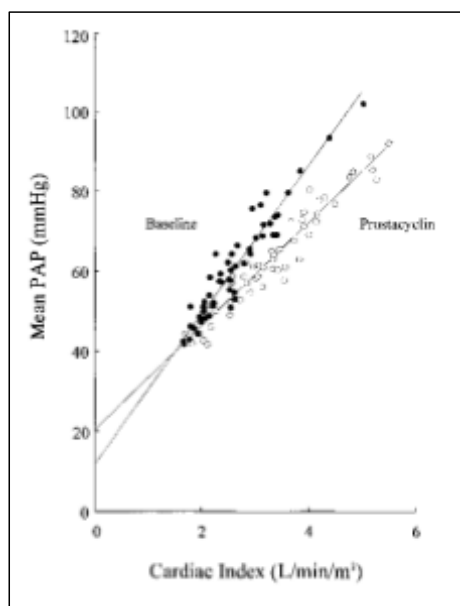


Figure 4: Relationship between mPAP and cardiac index in iPAH patients at baseline and after 6 weeks IV prostacyclin therapy. The pressure-flow slope declined after therapy despite no significant change in resting pulmonary haemodynamics but a significant improvement in exercise capacity.

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Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society, Castelain V, Chemla D, Humbert M, Sitbon O, Simonneau G, Lecarpentier Y, Herve P. Pulmonary artery pressure-flow relations after prostacyclin in primary pulmonary hypertension. *American journal of respiratory and critical care medicine*. 2002;165:338-40. *The American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society.

Exercise Doppler echocardiography provides an attractive method to non-invasively assess exercise pulmonary haemodynamics [46, 47] but is constrained by technical limitations (e.g. sonographer-dependence, difficulty obtaining appropriate acoustic windows, particularly of the RV); a paucity of data regarding the normal TRV during exercise and the influence of age, gender, and body mass; concerns regarding the precision and accuracy of PAP estimation from the TRV [27]; lack of a standardized exercise protocol; and, an incomplete understanding of the natural history of eiPH (i.e. the risk of incident PAH, and potential influence of heterogeneity depending on the underlying aetiology) [20, 48]. Consequently, TTE is not suitable as a stand-alone tool for baseline or follow-up assessment of RV function and haemodynamics in PAH, and exercise Doppler echocardiography is not recommended by guidelines for PAH screening [7].

In summary, pathophysiologic rationale for exercise testing patients at risk of latent or early PVD, with a view to detecting an ‘abnormal’ pulmonary vascular haemodynamic response, is sound but limited by difficulties standardizing the exercise protocol (introducing heterogeneity between patients and centres); accurately and reproducibly measuring haemodynamic changes non-invasively during exercise; defining an ‘abnormal’ pulmonary vascular response; and an incomplete understanding of the natural history of eiPH, which in part is related to the preceding limitations which introduce significant heterogeneity to available data. Rationale for the novel, non-invasive pulmonary vasoreactive challenge

assessed in the present body of work is grounded in addressing some of these limitations. The following sections will discuss and justify the chosen imaging modality, measurement parameters of interest, and method for ‘stressing’ the cardiopulmonary unit.

1.2.3 A NOVEL APPROACH

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (CMR), first utilised in PH more than 30 years ago [49], has become the gold-standard non-invasive method to measure RV structure and function in PAH [50, 51] with applications in diagnosis, risk assessment, and monitoring of disease progression/treatment response. Unlike TTE, CMR permits reliable, reproducible, and accurate morphologic and volumetric assessment of the RV with minimal operator dependence, no interference from adjacent bone or air, and no reliance upon geometric assumptions. The mechanical properties and blood flow of the proximal pulmonary vessels can also be examined using velocity-encoded sequences, allowing comprehensive assessment of the heart and pulmonary circulation (cardiopulmonary unit) in a single sitting. CMR is therefore a powerful tool for the non-invasive assessment of PVD and, while its use has thus far been restricted to the examination of PAH patients, its advantages make it an exciting potential tool for the detection of early PVD.

CMR-derived measurement parameters of interest

Initial remodelling of the RV is an adaptive response to an elevated afterload (AL) in the context of PVD, with hypertrophy serving to reduce wall tension (in accordance with the LaPlace law). RV mass can be calculated with CMR by multiplying myocardial volume by specific gravity ($1.05\text{g}/\text{cm}^3$), with superior reproducibility compared with TTE [52, 53]. In early stages of established PAH, RV mass correlates well with PAP [54] suggesting it may be

an early marker of PAH. However, RV mass can increase as a normal physiologic response to physical activity in healthy subjects, making it difficult to differentiate between physiologic and pathologic adaptation in asymptomatic individuals. Furthermore, like RV dilation and systolic dysfunction, RV hypertrophy is a relatively late phenomenon in the context of PVD, making it unsuitable for early disease detection.

It is often considered a drawback of CMR that the TRV cannot be measured and used to estimate pulmonary pressures non-invasively. However, unlike TTE, CMR allows the accurate and reliable measurement of blood flow velocity, blood volume, and blood vessel dynamics (e.g. pulsatility) via velocity-encoded phase-contrast cine CMR imaging (PC-CMR) through the main pulmonary artery (PA), producing two sets of cine images: *magnitude images* that provide anatomic information and *phase images* in which velocity information is encoded. Since the entire CO passes through the PA with each stroke before reaching the microvascular level where PVD predominates, PC-CMR at the PA level may provide ‘upstream’ insight to a ‘downstream’ vasculopathic disease. Two promising CMR indices are proximal pulmonary vessel dynamics (or stiffness) and pulmonary arterial blood flow velocity.

Proximal pulmonary vessel dynamics

The pulmonary circulation receives the entire cardiac output with each stroke so that during stress (e.g. exercise), the pulmonary vessels must distend to accommodate increases in blood volume and flow. The healthy pulmonary circulation is therefore a high compliance, low resistance system [55] that allows proximal vessels to buffer pulsatile flow and provide constant flow distribution downstream in an efficient manner. The afterload (AL) – the hydraulic load imposed on the RV during ejection [55] - is traditionally measured in terms of

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PVR, which describes the steady, resistive load (ratio of *mean* pressure to *mean* flow) only and neglects the pulsatile nature of blood flow. Therefore, the compliance (change in volume for a given change in pressure) of the pulmonary vasculature, or its ability to dilate to accept pulsatile stroke volume, needs also to be considered when describing the total load on the RV. This pulsatile component of RV AL has been shown to contribute approximately 25% of total AL in the pulmonary system in contrast to ~10% in the systemic system [56].

Reduced global arterial compliance and increased local proximal pulmonary arterial stiffness have both been demonstrated in PAH, and have been shown to be predictors of mortality, response to vasoreactivity testing, and functional capacity as determined by 6MWD [57]-[10, 58-63]. Histological specimens demonstrate changes in elastic pulmonary arteries of PAH patients that are likely to directly affect vascular compliance, including an increased burden of fibrosis and atypical elastic patterns [64]-[65]. Whether the increased stiffness is secondary to the elevated distending pressure alone, or pressure-independent changes of the vessel properties, is not yet fully elucidated [63]. However, there is mounting evidence implicating mechanical alterations in these vessels playing a pathophysiological role in disease progression, even in the early stages. Given ~~many of~~ these measurements can be made non-invasively by CMR, they form an attractive target of interest for the detection and monitoring of PVD.

The relationship between the resistive afterload (or resistance, R) and pulsatile afterload (or compliance, C) has been shown to be inverse and their product, the RC-time (describing the decay of PAP during diastole) the same in healthy individuals and patients with pulmonary hypertension (Figure 5) [66]. Lankhaar *et al* showed this relationship was maintained during therapy in patients with pre-capillary pulmonary hypertension, concluding that greater

haemodynamic improvement is achieved by a reduction in both R and C rather than R alone, which is a more common measure of treatment success [60]. This provides a plausible explanation for why patients with mild pulmonary hypertension (less severe R) often show greater clinical response to therapy than those with severe pulmonary hypertension (more severe R), despite a similar reduction in R [60, 67]. Furthermore, the inverse relationship between R and C highlights the importance of C on total RV afterload, implying that early in disease progression C may be compromised despite a normal R , which may explain why C is a strong predictor of survival and may be a suitable, non-invasive marker of early disease [60].

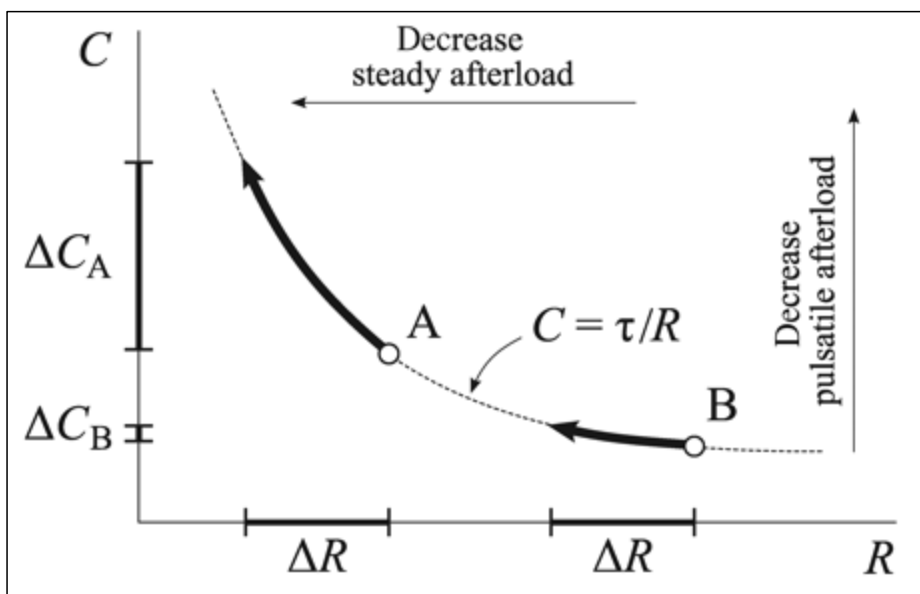


Figure 5: If the RC-time is constant, patients will always move along the curve so for a change in resistance ΔR , there will also be a change in compliance ΔC . Patient A, with mild pulmonary hypertension (lower baseline R), will have a greater ΔC for the same ΔR than

patient B, with more severe pulmonary hypertension (higher baseline R). Reprinted with permission, source [60].

Sanz *et al* assessed pulmonary arterial stiffness in 94 patients with known or suspected pulmonary hypertension using CMRI and same-day RHC data [63]. Three groups were identified based on RHC data: no pulmonary hypertension, exercise induced pulmonary hypertension (eiPH, mPAP >30mmHg with dynamic exercise), and pulmonary hypertension at rest (mPAP >25mmHg). They found strong correlation between increased PA stiffness, increased pulmonary pressures, and high PVR. This held true when adjusting for the distending pressure using stiffness index β . Interestingly, subjects with eiPH had lower arterial compliance and capacitance than those with no PH, despite similar resting haemodynamic profiles (mPAP, PVR etc.). acknowledging the limitations here of defining eiPH by the absolute mPAP value rather than the mPAP/CO relationship, and the lack of longitudinal data regarding clinical outcomes of eiPH, these findings suggests that alterations in PA elasticity may occur before the development of a measureable PAP rise, -a notion supported by the RC-time curve [43]-[60, 63].

This hypothesis is also biologically plausible. Stiffer proximal vessels can may directly contribute to disease progression by enhancing energy transmission to the distal vessels and microvasculature, altering shear stress and influencing endothelial cell function, vasoconstriction, smooth muscle cell proliferation and vascular remodelling [63, 68]. In addition to shear stress, stiffer proximal vessels can also affect downstream flow pulsatility. Li and colleagues recently looked at the specific effect of increased flow pulsatility caused by stiffer conduit vessels on a model of bovine pulmonary microvascular endothelial cells (ECs) [69]. High flow pulsatility, as compared to static or steady flow, significantly upregulated

gene expression for adhesion molecules and inflammatory cytokines implicated in PAH pathogenesis (e.g. ICAM-1, E-selectin, monocyte chemoattractant protein-1). Functional upregulation was confirmed by demonstration of increased monocyte adhesion in pulse flow conditions.

The effect of distal arterial EC dysfunction – caused by increased flow pulsatility - on smooth muscle cell (SMC) phenotypic changes has more recently been investigated using bovine pulmonary arterial co-culture (endothelial and smooth muscle cells, separated by a microporous membrane) [70]. In the presence of ECs, high pulsatility flow increased both SMC size and expression of the contractile proteins smooth muscle α -actin, and smooth muscle myosin heavy chain. When ECs were absent from the culture, high pulsatility flow decreased the expression of both contractile proteins without influencing the size or number of SMCs. Flow rate and shear stress were similar in the high pulsatility and control arms (steady flow and static flow), suggesting the pulsatile nature of the flow was responsible. Possible contributory EC-derived molecular signals were sought by analysing protein and/or mRNA expression. Vasoconstrictors [angiotensin converting enzyme (ACE), and endothelin-1 (ET-1)], vasodilator [endothelial nitric oxide synthase (eNOS)], and growth factor [transforming growth factor – β (TGF- β)] levels in ECs were compared. High pulsatility flow reduced eNOS, and increased ACE, TGF- β , and ET-1, which are frequently elevated in PAH patients. Pharmacological antagonism of these signalling pathways prevented pulsatility-induced EC-mediated SMC changes. Thus, a vicious cycle of remodelling can be proposed where proximal vascular stiffening augments downstream shear stress and pulsatility, promoting small vessel remodelling. In turn PAP rises, increasing circumferential stress on proximal vessels with subsequent smooth muscle cell-mediated

wall thickening and higher vessel stiffness, promoting further downstream remodelling and so on [71, 72].

These data suggests that non-invasive assessment of PA stiffness at rest may enable detection of early PVD. Based on the RC-time curve, it may also be possible to monitor change in PA stiffness under different loading conditions (e.g. in response to a vasodilator stress), thereby unmasking abnormalities not readily evident at rest. This is discussed later.

Pulmonary arterial blood flow velocity

Studies have identified CMR-derived parameters relating to mPAP [54, 63, 73-79] and PVR [80-83]. Recently Swift *et al* derived predicted mPAP from multivariate regression analyses of CMR measurements taken from 64 treatment-naïve patients with suspected pulmonary hypertension [84]. They went on to validate this in another 64 patients, as well as estimate surrogate measures of PAWP and CO. Estimated pulmonary vascular resistance was calculated from these measurements. A strong correlation between CMR-estimated mPAP and RHC-derived mPAP was found ($R^2=0.67$), and CMR could reliably identify a $PVR \geq 3$ Woods Units (area under ROC curve = 0.94, CI 0.88-0.99 p value <0.0001). However, the statistical model for estimating mPAP is cumbersome and is not validated in treated patients or under different loading conditions, such as during vasoreactivity testing.

A more straightforward PA flow parameter to measure from phase-encoded CMR images is the mean pulmonary arterial flow velocity (meanPAvel), which has been shown to strongly correlate with PVR and mPAP in an inverse logarithmic manner [78, 81-83]. Postulated contributing mechanisms to this inverse correlation include small vessel remodelling,

vasoconstriction, and in situ thrombosis slowing flow through these vessels; PA dilatation in the setting of chronic pressure overload; and RV systolic failure in more advanced disease [82]. By averaging the velocities through the entire cardiac cycle rather than just systole, as is measured by Doppler echocardiography, the assumption that flow is uniform during all phases is avoided [83]. In 2007, Sanz and colleagues sought to identify PA flow parameters obtained by phase-encoded MR that may allow non-invasive diagnosis of PH [78] and of the many flow parameters assessed, meanPAvel showed the strongest correlation with invasively acquired haemodynamic measurements, with correlation coefficients of -0.86, 0.76, and -0.73 for PVRI, sPAP, and mPAP respectively. These did not vary significantly among different subgroups of PH, although interpreting values in high output states (e.g. portopulmonary syndrome) was cautioned.

Garcia-Alvarez *et al* showed meanPAvel to be the strongest univariate predictor of PVR in their derivation of a non-invasive estimation of PVR, and hence it carried the highest weight in that predictive model (Spearman correlation coefficient of -0.83 between meanPAvel and PVR) [82]. The relationship demonstrated between meanPAvel and PVR was curvilinear (Figure 6) and therefore at very high PVRs, meanPAvel may be a less accurate reflection of PVR [82]. Nevertheless, this shouldn't preclude detecting changes over time, such as a reduction down to moderately elevated PVR levels – an important indicator of treatment response. Indeed, the same laboratory went on to show that the change in MRI-derived parameters correlated with haemodynamic changes over time in PAH patients [81]. Patients with haemodynamic improvement had increased meanPAvel and reduced PA area, whilst those that did not improve had reduced meanPAvel and increased PA area. Consequently, these CMR-derived parameters were found to have a good ability to identify haemodynamic improvement (area under receiver operating characteristic curve of 0.83, $p=0.014$, for

meanPAvel; and 0.81, $p=0.021$, for PA area). Garcia-Alvarez *et al* also demonstrated the ability of PA velocity measurements to reflect changes in PVR over time, this time using a porcine model of PAH induced by microsphere embolization [83]. They estimated a 10% decrease in meanPAvel correlated with a 1 Wood unit rise in PVR. Finally, the same laboratory showed a strong correlation between change in meanPAvel and change in PVR and mPAP during acute vasodilator testing with 100% oxygen in the same porcine model, suggesting this could be a method of non-invasively assessing vasoreactive response [83].

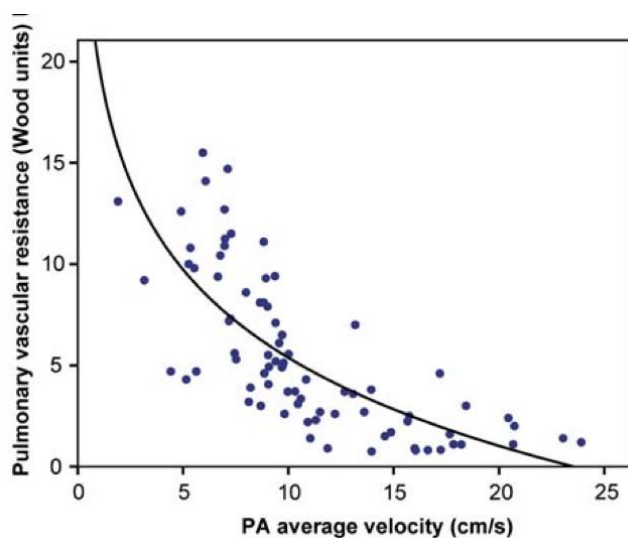


Figure 6: Univariate association of PVR and meanPAvel. Reprinted with permission, source [82].

'Stressing' the cardiopulmonary unit

Like most stress tests, dynamic exercise assessment of the cardiopulmonary unit in the context of PVD is likely to provide pathophysiological insight not necessarily evident at rest. As discussed, widespread application is hindered by difficulty standardising the exercise

stress component and non-invasively measuring appropriate physiologic parameters and, while exercise testing during CMR is possible [85, 86], it is technically and logistically difficult and not widely available. An alternative approach is to use a vasodilator agent as a stress modality, a practice commonly employed during diagnostic RHC of PAH patients (invasive vasoreactive challenge). Adenosine is a readily available endogenous nucleoside analogue with potent vasodilator properties that has been used in PAH as a vasoreactive agent for over 20 years, and is an accepted agent for this purpose in current guidelines [40]. Administered intravenously, it has selectivity for the pulmonary circulation given its rapid metabolism and short half-life [87]. Furthermore, its predominant effect of microcirculatory vasodilation does not require an intact endothelium [88, 89], and in higher primate models, no effect on conduit pulmonary vessel diameter has been found [90] [89]. Previous studies using adenosine in PAH have shown a significant reduction in PVR (33% - 39%) associated primarily with elevated cardiac output rather than reduced mPAP, and largely unchanged mean systemic arterial pressure [87, 91, 92]. These properties, its favourable safety profile, and its frequent use as a coronary hyperaemic agent both invasively and non-invasively make adenosine an attractive agent for use as a 'stress' agent in this population, particularly given the administered doses can be easily controlled and replicated [93].

Rationale for the use of adenosine as a vasodilatory stress agent for the present protocol stems from pre-clinical and clinical studies addressing the prognostic relevance of invasive haemodynamic changes during vasoreactive challenge in established PAH, and the concept of pulmonary flow reserve (PFR), an analogous measure of pulmonary microcirculatory compromise to coronary flow reserve (CFR), a commonly employed and well validated marker of coronary vascular integrity. These concepts, and how they may be measured with CMR, will be discussed in the ensuing sections.

Commented [TB1]: Adenosine

Invasive pulmonary vasoreactivity in established PAH

Patients with PAH are often challenged with a vasoreactive agent during diagnostic RHC to determine suitability for calcium channel block therapy. The traditional 'positive response' is near normalisation of pulmonary pressures (decrease in mPAP by ≥ 10 mmHg to ≤ 40 mmHg) with an unchanged or increased CO [94]. Only ~10% of iPAH and APAH patients exhibit this 'classic' response, and approximately half of these show sustained clinical response to CCB therapy [95]. However, this challenge can also provide pathophysiological insight if considered on a continuum rather than as an all-or-nothing outcome, with larger improvements in haemodynamics possibly reflecting a phenotype with more vasoconstriction and less vascular remodelling [96]. Malhotra *et al* retrospectively assessed the utility of using the response to inhaled nitric oxide (NO) and oxygen to predict survival in 80 newly diagnosed PAH patients [97]. Median reduction in PVR was 30%, and median reduction in mPAP was 12% with inhaled NO/oxygen. Baseline characteristics and haemodynamics of patients stratified above and below these median values were similar. Dichotomised, those with $\geq 30\%$ reduction in PVR had a 53% reduction in age-adjusted mortality, and those with $\geq 12\%$ reduction in mPAP had 55% reduction in mortality over a median follow up period of 2.4 years. This held true after adjusting for known multivariate predictors of survival, such as age and right atrial pressure (RAP).

McLaughlin *et al* found the change in PVR with intravenous adenosine was a univariate predictor of survival in PAH patients going on to intravenous epoprostenol therapy [98]. In contrast, baseline mPAP and PVR did not predict survival. A more significant PVR reduction ($\geq 50\%$) in response to short-term prostacyclin challenge was also found to be a

Commented [TB2]: Vasoreactivity

univariate predictor of mortality in a retrospective study by Raffy *et al* [99]. Again, baseline haemodynamics were similar and unable to predict survival. A recent retrospective analysis of iPAH patients diagnosed in a single centre in Korea showed that baseline vasoreactive response was a good predictor of survival, whereas baseline rest haemodynamics were not (only mPAP was significantly different between ‘responders’ and ‘non-responders’ at rest) [100].

This work highlights the potential utility of vasoreactive testing to not only identify the small number of patients that may benefit from CCB therapy, but also to aid in prognostication and patient management (e.g. more aggressive drug therapy or closer patient monitoring in ‘poor’ responders). While the pathobiologic processes that govern vasoreactivity are incompletely understood [101], a rational hypothesis is that compared with resting measures, haemodynamic changes during vasoreactivity challenge more accurately define vasculopathic burden and hence, cardiopulmonary reserve (*NOTE*: not considering those with a ‘true’ vasoreactive response and sustained therapeutic benefit with CCB therapy: these patients likely represent a distinct molecular pathophenotype [101]). This approach has not been applied to the detection of early PVD, in part due a lack of non-invasive modality with which to measure response. Changes in proximal pulmonary vessel dynamics and meanPAvel measured with CMR may provide such a method.

Vasoreactive challenge and pulmonary vessel dynamics

The relationship between vasoreactivity response, haemodynamic parameters, and intrinsic elastic PA properties has been investigated invasively using intravascular ultrasound (IVUS) of elastic pulmonary arteries (2-4mm) during RHC in patients with iPAH [102]. This study demonstrated that patients who vasodilated during epoprostenol infusion (vasoreactive agent)

as measured by IVUS (increase in diastolic area, $dA \geq 10\%$) had higher baseline IVUS pulsatility (IVUSp) and lower baseline elastic modulus (E). This was despite similar resting mPAP and pulse pressure between both groups, implying the reduction of IVUSp and E were due to differences in intrinsic viscoelastic properties of the arteries rather than haemodynamic conditions. However, there was no correlation between baseline arterial stiffness, vasodilator response (dA change) and vasoreactive response as measured by haemodynamic changes (mPAP, PVR). Those that did vasodilate (IVUS $dA \geq 10\%$) had a stable mPAP but increased CI, capacitance, and stroke volume index, and therefore a reduced PVR [102]. This is in keeping with the RC-time constant discussed previously, and raises the possibility that assessing elastic arterial vessel properties (i.e. pulsatile afterload) during vasoreactive testing may improve identification of patients with less severe remodelling, more vasoconstriction and possibly improved response to vasodilator therapies on the basis that a 'vasodilator patient' may not be a 'vasoreactive patient' as assessed by currently recommended haemodynamic parameters.

Local pulmonary artery mechanical properties have also been investigated before and after six months of bosentan therapy (an endothelin receptor antagonist) in a small number of PAH patients, again using IVUS and RHC data [103]. At baseline, PAH patients had thicker arterial walls ($34\% \pm 2\%$ vs. $17\% \pm 2\%$) despite similar luminal diameters, reduced compliance, reduced distensibility, higher elastic modulus and higher stiffness index β compared with age and sex matched controls. The higher stiffness index β and elastic modulus implied altered intrinsic mechanical properties secondary to vascular remodelling, rather than simply a manifestation of the altered haemodynamic conditions. Following 6 months of bosentan therapy, RHC with IVUS did not show a change in any markers of pulmonary artery stiffness or wall thickness. Ignoring the limited power of this study ($N = 8$

patients), this could be explained by the fact that severe PAH patients such as those included (average mPAP 48mmHg \pm 3mmHg) operate on the flat and stable part of the distensibility/mPAP curve (Figure 5), and since the RC-time constant remains the same during therapy, a relatively large reduction in PVR would be required to modify arterial compliance. Alternatively, it may be all 8 patients were ‘non-responders’ by traditional markers of treatment response (e.g. haemodynamic data or clinical indicators), although these data were not presented to confirm or refute this. This raises the possibility that less severe PAH patients (lower baseline mPAP), or severe PAH patients that respond to therapy (e.g. increased functional capacity, reduced PVR) may show changes in pulmonary stiffness with therapy. The corollary is that pulmonary vessel stiffness precedes increased PAP and PVR, and therefore PAH-specific therapy may not affect pulmonary stiffness in advanced disease.

An increase in proximal PA compliance, measurable by CMR, may therefore accompany a temporary reduction in the resistive AL (or PVR) induced by IV adenosine (leftward shift on the RC curve, Figure 5). This may enable a more comprehensive assessment of the pulmonary vasculature than possible by resting measures (RHC or CMR-derived) alone, thereby facilitating detection of early PVD or, in established PAH, providing a novel means to monitor disease progression and treatment response longitudinally.

The concept of pulmonary flow reserve and relationship to CMR-derived meanPAvel measured during vasoreactivity

The concept of flow and haemodynamic assessment under different loading conditions is not new but there is a paucity of data relating to its application in the assessment of the pulmonary circulation. Coronary flow reserve (CFR) is a validated measure of the functional status of the coronary circulation, and is derived by assessing Doppler flow velocity at rest

and with hyperaemia, often induced with adenosine [104]. It reflects the magnitude of the increase in coronary flow above baseline when maximal coronary vasodilation is present. Given most resistance to flow is microvascular in origin, in the absence of epicardial coronary artery stenoses, it is a marker of microvascular function. Indeed, lower CFR values have been associated with increased cardiovascular events [105]. In the pulmonary circulation, the small arteries and arterioles are also a key site of resistance, since resistance is strongly related to vessel diameter [106].

Commented [TB3]: CFR/PFR

At present, there is no validated method to assess pulmonary microcirculatory function in humans. Ilsar and colleagues used a higher primate model to investigate the feasibility of measuring the physiological index of 'pulmonary flow reserve' (PFR = maximal hyperaemic pulmonary blood flow/basal pulmonary blood flow) [90]. They used a Doppler sensor guidewire advanced into segmental level pulmonary arteries of ketamine-anaesthetised baboons. Saline, papavarine and adenosine were infused at incremental doses into the pulmonary circulation and vessel diameter, flow velocity and haemodynamics were recorded pre- and post- infusion. They found that measurement of PFR in this way was feasible and reproducible using either adenosine or papavarine (no change in Doppler velocity was seen with saline infusion). Importantly, adenosine produced dose-dependent incremental hyperaemia that plateaued at 200mcg/kg/min, and there was no change in vessel diameter observed. Therefore, they concluded that in vivo measurement of PFR may have a role in the assessment of pulmonary microcirculatory integrity.

Ilsar *et al* went on to demonstrate that PFR could track cumulative microvascular obstruction in a baboon model [107]. Pulmonary flow reserve was again measured with a Doppler sensor guidewire using intrapulmonary adenosine infusion at a dose of 200mcg/kg/min to induce

hyperaemia. Ceramic microspheres were then infused in increasing amounts (10^4 , 10^5 and 10^6 particles) into the catheterised pulmonary arterial segment. Pulmonary flow reserve was re-calculated at each dose of microspheres and a progressive reduction in Doppler flow velocity with increasing particle administration was noted. Resting Doppler velocity was not affected until the highest dose of ceramic microspheres, suggesting PFR is sensitive to microcirculatory changes of insufficient severity to affect resting flow. However, generalizability of these findings is limited by the small sample size (3 baboons) and lack of validation of microsphere obliteration as a PAH model.

The same laboratory conducted a similar study on 13 baboons, but instead used thermodilution-derived mean transit-time (T_{mn}) as a surrogate for pulmonary blood flow (instead of Doppler-derived flow velocity), allowing the calculation of both PFR and Pulmonary Index of Microcirculatory Resistance (PIMR = maximum hyperaemic distal pulmonary artery pressure \times maximum hyperaemic T_{mn}) [107]. In the coronary circulation, the index of microcirculatory resistance (IMR = maximum hyperaemic distal coronary artery pressure \times maximum hyperaemic T_{mn}) has been shown to be more reflective of true microcirculatory resistance than CFR, which also incorporates epicardial coronary artery integrity [108-110]. Using a similar protocol to previous studies, PFR_{thermo} and PIMR were calculated at rest and with progressive microvascular obliteration induced by microsphere infusion. Feasibility and reproducibility of both measurements were demonstrated. In response to incremental microvascular obliteration, PAP remained unchanged but a progressive reduction in PFR_{thermo} , and increase in PIMR was seen (see figure 7). Again, this provides evidence that assessment of pulmonary microcirculatory integrity may provide a novel technique to identify pulmonary vascular disease before measurable PAP or PVR elevation, although human studies are required.

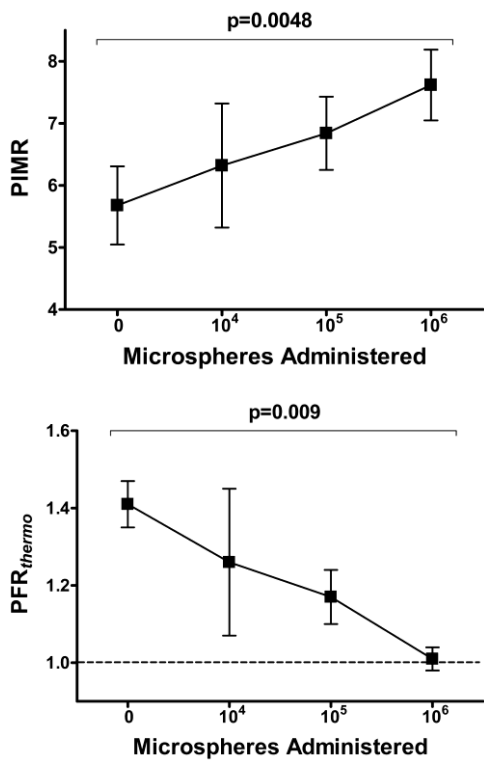


Figure 7: Cumulative microvascular obstruction induces progressive reductions in PFR_{thermo} and increases in PIMR.

Reprinted with permission, source [111], Fig. 2.

While invasive PFR has not been validated in humans, resting pulmonary flow velocity waveforms have been studied using Doppler sensor guidewires [112]. These were advanced

into the distal segment of the left lower lobe pulmonary arteries of 7 treatment-naïve PAH patients, both before and after 6 months of PAH-specific therapy (bosentan). Importantly, the waveforms obtained had excellent signal-noise ratio, implying a feasible technique. Treatment responders (n = 3) and non-responders (n = 4) were defined by the presence or absence of >40m increase in 6-minute walk distance and an improvement in functional class. Responders showed a large increase in average diastolic velocity as well as mean velocity of pulmonary blood flow over the 6-month period. Presumably this reflected a reduction in distal vascular resistance, although haemodynamics were not reported. Nevertheless, it does provide the basis for further study to investigate pulmonary blood flow assessment can provide insights into the pulmonary vasculature, and how this may assist with diagnosis, prognostication and monitoring of treatment response.

Assessment of coronary microcirculatory integrity can be confounded by the presence of epicardial coronary artery stenosis. In this setting, a low CFR as assessed by Doppler flow velocity can reflect epicardial coronary stenosis, microvascular dysfunction, or a combination of both. The IMR, after collateral flow is considered, is more specific for coronary microvascular integrity as it is not impacted by epicardial stenosis [108]. In contrast, the pulmonary circulation receives the entire cardiac output with each stroke via the main pulmonary artery and, except for chronic thromboembolic pulmonary hypertension (CTEPH) with proximal thrombus, pulmonary flow is not impeded in pulmonary vascular disease until the proximal microcirculatory level where pathognomonic remodelling occurs. Using intravenous adenosine as a hyperaemic agent and acknowledging that intravenous adenosine has been shown to preferentially vasodilate the pulmonary circulation over the systemic circulation (presumably due to the rapid metabolism limiting systemic drug delivery) [93, 113]; CO is thought to increase as a direct result of reduced RV afterload rather than

enhanced inotropy [93, 114, 115]; pulmonary vascular disease in PAH is heterogeneous in anatomical location throughout the lungs [116, 117]; and the site of vasodilation is thought to be the proximal microcirculation where remodelling predominates [90, 111], it can be hypothesized that PFR measured by blood flow velocity (assuming unchanged vessel cross sectional area) at any point 'upstream' will reflect pulmonary microcirculatory integrity and the ability of the RV to augment flow in proportion to AL reduction. Acknowledging the superior prognostic power of clinical and haemodynamic parameters that reflect functional reserve over those measured at rest, this should provide a more comprehensive assessment of the RV/pulmonary arterial system than resting parameters alone [118-120]. Furthermore, acknowledging that CMR-derived meanPAvel correlated strongly with change in PVR and mPAP during acute vasodilator testing in a pre-clinical study, this may be a feasible parameter with which to quantify the vasoreactive response and pulmonary blood flow changes non-invasively in humans [83].

1.2.4 RISK ASSESSMENT IN ESTABLISHED PAH

Periodic longitudinal evaluation of PAH patients to assess severity, prognosis, and treatment response is recommended by current guidelines [7]. Since no single investigation is sufficiently instructive, the recommended approach is multidimensional incorporating clinical, echocardiographic, exercise, haemodynamic, and biomarker assessments (Figure 8). Risk stratification, based on estimated 1-year mortality (low risk <5%; intermediate risk 5-10%, high risk >10%), is applicable at the time of diagnosis as well as during follow-up, with low risk criteria functioning as treatment goals. There are limitations to this approach: many cut-offs are based on low level evidence and expert opinion; it is resource intensive; and, prognostic relevance of these parameters at follow-up are less well understood, as are their

changes longitudinally. A unified approach would simplify patient management and therapeutic decision-making.

Determinants of prognosis* (estimated 1-year mortality)	Low risk <5%	Intermediate risk 5–10%	High risk >10%
Clinical signs of right heart failure	Absent	Absent	Present
Progression of symptoms	No	Slow	Rapid
Syncope	No	Occasional syncope ^b	Repeated syncope ^c
WHO functional class	I, II	III	IV
6MWD	>440 m	165–440 m	<165 m
Cardiopulmonary exercise testing	Peak $\dot{V}O_2 > 15$ ml/min/kg (>65% pred.) VE/ $\dot{V}CO_2$ slope <36	Peak $\dot{V}O_2$ 11–15 ml/min/kg (35–65% pred.) VE/ $\dot{V}CO_2$ slope 36–44.9	Peak $\dot{V}O_2 < 11$ ml/min/kg (<35% pred.) VE/ $\dot{V}CO_2$ slope ≥ 45
NT-proBNP plasma levels	BNP <50 ng/l NT-proBNP <300 ng/ml	BNP 50–300 ng/l NT-proBNP 300–1400 ng/l	BNP >300 ng/l NT-proBNP >1400 ng/l
Imaging (echocardiography, CMR imaging)	RA area <18 cm ² No pericardial effusion	RA area 18–26 cm ² No or minimal, pericardial effusion	RA area >26 cm ² pericardial effusion
Haemodynamics	RAP <8 mmHg CI ≥ 2.5 l/min/m ² SvO ₂ >65%	RAP 8–14 mmHg CI 2.0–2.4 l/min/m ² SvO ₂ 60–65%	RAP >14 mmHg CI <2.0 l/min/m ² SvO ₂ <60%

Figure 8: Risk assessment in PAH.

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Since PAH is, by definition, a haemodynamic condition, it is not surprising that the prognostic relevance of invasive haemodynamic parameters have received most attention. Haemodynamic markers of RV function at baseline – right atrial pressure, cardiac index and cardiac output - have been shown to be predictors of survival [14, 121]. Pulmonary vascular resistance and mPAP have also been shown to be baseline prognostic indicators [121, 122], although mPAP can decline in advanced disease with RV dysfunction and, therefore, variables that directly reflect RV function generally have superior predictive value [40].

Non-randomised studies have reported associations between longitudinal haemodynamic changes and clinical events (65, [98, 123–125]). Improvements in cardiac index have predicted prognosis, emphasising the importance of RV function and adaptation on survival [98, 124, 126]. A reduction in PVR at different time points after treatment initiation has also been

associated with better survival independent of the therapy instituted [98, 123-125]. A retrospective analysis of 122 PAH patients with repeat RHC 3-4 months after treatment initiation addressed the long term predictive value of early changes in haemodynamic parameters [127]. An increase in CO of $\leq 0.22\text{L}/\text{min}$ was associated with a significantly worse transplant-free survival over a mean follow up time of 4.7 years, with a hazard ratio of 2.05 (CI 1.15-3.67, p value 0.015). A larger reduction in PVR was also a predictor of better transplant-free survival. Dichotomised into higher and lower PVR reductions, the latter group had an adjusted HR of 1.89 (CI 1.02-3.51, p value 0.044) for transplant or death (HR adjusted for date of diagnosis, diagnostic class, age and gender). The same was not seen for changes in mPAP, with an adjusted HR for those dichotomised to a lower reduction group of 1.12 (CI 0.63-1.97, p value 0.704) for transplant or death.

A more recent meta-analysis investigated the relationship between haemodynamic changes and clinical events (all-cause death, hospitalisation for PAH, lung-heart transplant or initiation of rescue PAH therapy) based on data from randomised clinical trials [128]. Sixteen randomised trials were included with a total of 2353 patients. They demonstrated that pharmacological treatment was associated with a significant reduction in clinical events. A significant inverse correlation was found between change in 6MWD and change in PVR and CI. However, no relationship was found between the change in haemodynamic parameters (PVR, PAP, CI and RAP) and clinical events when analysed as a composite or individually. It should be noted however that follow-up duration in these trials was short (usually 3-4 months) and therefore it is unknown whether longer-term follow-up would yield different results.

Venetuolo *et al* undertook a patient-level pooled analysis of four therapeutic, randomised placebo-controlled trials to explore whether changes in haemodynamics measured 12-weeks after treatment initiation accounted for differences in clinical events (death, lung transplantation, hospitalisation for PAH, atrial septostomy, escalation of PAH therapy or withdrawal due to clinical worsening) [129]. Like the findings of Savarese *et al*, this analysis of 1119 patients found that treatment significantly reduced the odds of a clinical event and lowered RAP, PVR and mPAP, and increased CI. A decrease in PVR and an increase in CO/CI were associated with a significant reduction in the odds of a clinical event, while no such association was evident with change in mPAP or PA compliance. They went on to quantify the proportion of the treatment effect (reduction in clinical events) attributable to haemodynamic changes. At most, they found a change in CI and PVR accounted for 11.7-13.9% of the impact of treatment on event rates, and they were therefore unable to conclude that haemodynamic changes over short-term treatment periods could act as surrogate markers for clinical events. Acknowledging that these findings pertain to short-term follow-up periods only, the authors postulated that therapy may act on organ systems other than the pulmonary circulation to confer benefit.

Alternatively, treatment may alter RV loading conditions in a more complex manner than is assessable by rest haemodynamics alone, as suggested by Castelain *et al* [45]. Moreover, unrelated systemic processes such as anxiety, hypertension, and sedation may influence hmodynamic measures made at rest, and a single PVR measurement in PAH patients may be misleading due to the fact that the zero-flow intercept of a pressure-flow plot is positive and, therefore, the PVR is no longer a constant independent of the absolute blood flow and mPAP [45]. A more reliable assessment of the functional state of the pulmonary circulation may be achieved by examining the slope reflecting the multipoint relationship between CO and

mPAP (‘true PVR’) taken during incremental exercise [10, 45, 130]. To date no prospective therapeutic studies have incorporated the assessment of pulmonary haemodynamics under different loading conditions (e.g. graded exercise, repeat vasoreactivity challenge) over time with clinical event data other than exercise tolerance [45, 130]. The hypothesis that dynamic changes in pulmonary haemodynamics - or appropriate non-invasive surrogates - assessed under different loading conditions (i.e. ‘stress’) over time will provide more insightful prognostic data warrants further study. Acknowledging the prognostic relevance of haemodynamic changes during diagnostic vasoreactive challenge, and the prospect of measuring these changes with CMR-derived surrogates (proximal pulmonary vessel dynamics and meanPAvel), a standardized, non-invasive pulmonary vasoreactivity protocol using IV adenosine as the stress agent may afford such a means.

Cardiac magnetic resonance imaging meets many of the primary requirements for an ideal tool to monitor PAH patients: reproducible assessment of RV size and function, minimal observer and operator dependence, standardized, non-invasive, ‘patient-friendly’, time-efficient, and capable of providing prognostically relevant information at baseline and during follow-up. Regarding the latter, there is accumulating data supporting the prognostic value of the longitudinal change in several CMR-derived indices in established, treated PAH patients [131]. Van de Veerink and colleagues found RV ejection fraction (RVEF) was a stronger baseline predictor of survival than PVR, and change in RVEF was independently related to mortality whereas change in PVR was not, highlighting the importance of monitoring RV function [132]. Low stroke volume (SV) at baseline has been linked to poor survival, as has a lack of improvement in SV with PAH treatment, with change in SV also linked to exercise capacity as determined by 6-minute walk distance (6MWD) [133, 134]. The authors also noted a stronger link between survival and change in SV than change in cardiac index (CI),

presumably due to heart rate (HR) compensation [133], and calculated a minimal important difference in indexed SV of 10 ml at follow-up which correlated with a clinically relevant rise in 6MWD over the same period [134].

Right and left ventricular volumes have also been shown to independently predict survival at baseline, and progressive dilation of the RV and contraction of the LV over time has been related to functional decline and early mortality [133, 135]. A recent systematic review and meta-analysis addressing prognostic CMR parameters in PAH confirmed a rise in RV end diastolic volume (RVEDV) and RV end systolic volume (RVESV), as well as a decline in LV end diastolic volume (LVESV), predicted an adverse prognosis [136]. These findings highlight the inexorable link between the left and right heart chambers whereby maladaptive remodelling and systolic dysfunction of the pressure-loaded RV impacts LV geometry and filling, primarily via septal shift [137].

Change in RV mass does not seem to be closely linked to survival [133, 135]. Since correlation between PAP and RV mass has been demonstrated in mild PH [54], RV hypertrophy is likely an adaptive response to a pressure load. With further disease progression, RV dilation outstrips compensatory hypertrophy, explaining its poor prognostic relevance as a follow-up marker.

Less data exists regarding the prognostic value of change in proximal pulmonary vessel dynamics (i.e. PA stiffness) with therapy in established PAH. As already noted, low pulmonary vascular distensibility may be an early marker of PVD and has, as such, been linked to an adverse prognosis at baseline in established PAH [59, 61, 138]. Change in meanPAvel has not been evaluated in this context. Moreover, there is paucity of data

regarding CMR-derived indices measured during physiological stress, and no apparent studies evaluating change in such indices or their relationship to PAH prognosis, which may stem from limited capabilities of CMR centres to marry exercise stress and CMR. Forouzan *et al* reported the feasibility of performing supine exercise during CMR in 21 healthy subjects, demonstrating a rise in PA stiffness (determined using the flow area method to measure pulse wave velocity, a marker of PA stiffness) with incremental exercise load although no change in relative area change of the main PA (RAC, maximum cross-sectional area - minimum cross-sectional area/max cross-sectional area) was found [85]. The authors concluded that the protocol great potential in clinical practice to non-invasively assess the pulmonary vasculature.

As CMR protocols become more standardised and the associated costs decline, CMR is likely to become routine in the evaluation of established PAH. The capacity to study the pulmonary vasculature, RV, and their interaction in a single sitting is enticing. Furthermore, accessing the inherent benefits associated with physiologic stress may improve the yield of longitudinal evaluation significantly, and will be evaluated in the present body of work.

1.2.5 THE RIGHT VENTRICLE AND CORONARY BLOOD SUPPLY/DEMAND

Under normal loading conditions, early pre-clinical studies demonstrated that cardiac output (CO) and venous pressures were largely unaffected when the RV was rendered non-functional by cauterization or occlusion of the right coronary artery (RCA) [139-141], implying a conduit rather than contractile role. Pre-dating this work, Fineberg and Wiggers proposed that “circulatory failure following obstruction of the pulmonary circuit had no other

cause than fatigue of the right ventricle” [142]. While progress has been made to elucidate the mechanisms of RV fatigue in the context of an elevated AL, this has not translated to clinical treatments targeting the RV specifically, as has occurred in LV systolic dysfunction. Early pre-clinical work identified an increasingly important link between right coronary artery blood supply and cardiac performance with rising RV AL, although this relationship is not well described in humans.

Adaptive and maladaptive RV remodelling

Right ventricular geometry is complex, consisting of inflow (sinus) and outflow (conus) tracts separated by the membranous interventricular septum. Both the free wall and interventricular septum are convex (under normal loading conditions), so that the RV appears crescentic in shape in cross-section. Contraction is comprised of three patterns of myocardial deformation: inward or ‘bellowing’, circumferential, and longitudinal shortening, with longitudinal shortening contributing most to overall contractile performance [143]. The RV free wall is thin relative to the left ventricle (LV) because it uses only ~ 25% of the stroke work to pump the same stroke volume into the low resistance pulmonary circulation [144]. In the face of an elevated afterload, the RV must adapt to maintain forward flow and it is this capacity which primarily dictates prognosis in PAH. [14, 121, 122, 145]. Adaptation involves myocardial hypertrophy and increased contractility in the early stages but with PVD progression, adaptive responses become insufficient to overcome AL leading to chamber dilatation as a means to maintain preload and cardiac output. The interventricular septum flattens, reducing LV filling and stroke volume, although the ejection fraction is usually preserved [146]. In late stages, RV wall stress is increased as dilatation is incompletely matched by hypertrophy (law of Laplace). These changes are associated with an increased oxygen requirement.

Right ventricular remodelling associated with impaired systolic and diastolic function has been termed ‘maladaptive’, and predominates in PAH that is idiopathic in origin or secondary to connective tissue diseases. In contrast, RV remodelling can be associated with preserved RV function, and is characterised by a less dilated, more hypertrophied RV. This is termed ‘adaptive’, and can be present in PAH secondary to certain congenital heart defects (Figure 9) [147]. The complex pathobiology driving maladaptive remodelling of the pressure-overloaded RV is gradually being elucidated, but much remains unknown. At a cellular level, thick filament protein myosin heavy chain (MHC) undergoes subunit α – to β -isotype switch, which can impact deleteriously on systolic function of the RV [148, 149]. Myocyte thin filament changes are also seen in the stressed RV, with increased expression of α -skeletal actin and α -smooth muscle actin, and reduced expression of α -cardiac actin, although the impact on systolic function of these changes is not clear [149]. Proteins involved with regulating contractility, such as troponin and tropomyosin, are also implicated in the pathobiology of maladaptive remodelling, although most knowledge in this area is derived from scientific interest in left ventricular systolic dysfunction in left-sided heart failure. Left-sided models have also shown altered enzymes and ion channels directly involved with excitation-contraction coupling, depletion of adenosine triphosphate and other fuel sources, and a shift to glycolysis from fatty acid oxidation [149, 150]. Whether these events occur in the failing RV is unknown at this stage, although the metabolic shift to glycolysis is well documented in RV failure.

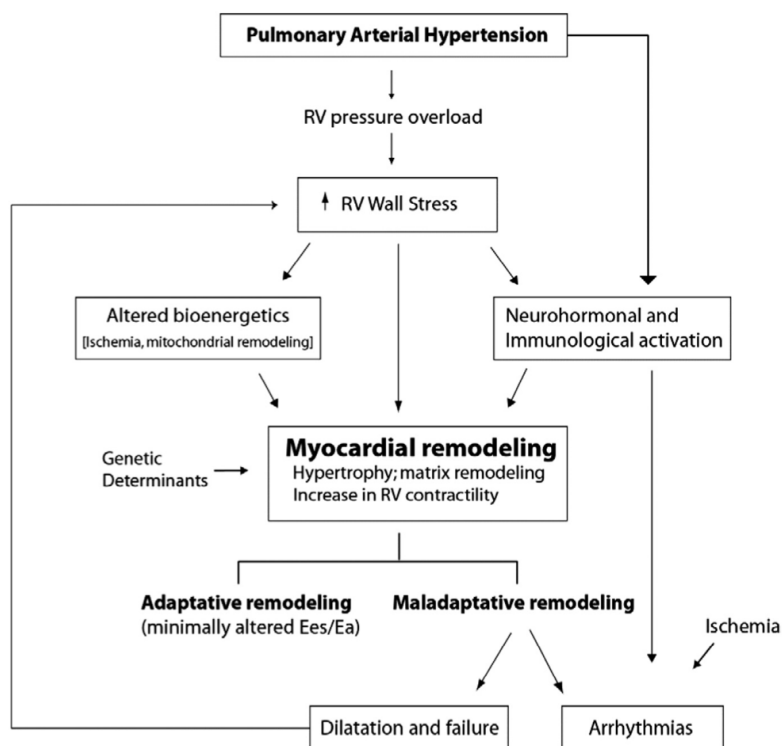


Figure 9: Pathophysiology of RV remodelling in PAH.

Reprinted with permission, source Vonk-Noordegraaf *et al.*, JACC Vol. 62, No. 25, Suppl D, 2013.

The extracellular matrix (ECM) is also implicated in maladaptive remodelling. Increased fibrosis has been demonstrated in myocardial biopsies of PAH patients, and experimental models have shown increased collagen content linked to TGF- β signalling, and excessive activity of matrix metalloproteinases (MMPs) [151, 152]. Fibrosis may be partly caused by RV ischaemia and the inability of the microcirculation to adapt to the hypertrophic response (e.g. inadequate capillarization) [153]. An inflammatory contribution has been postulated, with an increased density of mast cells and neutrophils observed in pressure-loaded RV histopathological specimens obtained from human and animal-model studies [154, 155].

Paracrine signalling from these cells may contribute to driving ECM degradation and scar deposition, thereby promoting ventricular dilatation and impaired compliance [78, 152, 156]. Interestingly, fibrosis in the pressure-loaded RV seems to occur less extensively than in the pressure-loaded LV (e.g. aortic stenosis, hypertension) [78, 156, 157], possibly explaining successful recovery of RV systolic function after lung transplantation for PAH, even when it is severely compromised at the time of transplant [158-160].

Modifying these pressure-related changes are various neurohormonal, oxidative, nitrosative, ischaemic, and apoptotic influences and adaptations. The renin-angiotensin system, adrenergic system, and natriuretic peptides are upregulated, with end-stage mediators (e.g. angiotensin II, aldosterone, and natriuretic peptides) reaching the RV via the systemic circulation. Local cardiac cells, such as myocytes, endothelial cells, and fibroblasts, are also capable of secreting neurohormones in a paracrine fashion. Via receptor tyrosine kinases (TKRs) and G protein-coupled receptors (GPCRs), they affect cardiomyocyte growth, proliferation, and survival [149]. In PAH, the net result in the RV is myocyte hibernation, contractile dysfunction, and growth arrest, which contrasts sharply with that in the pulmonary vascular bed where resistance to apoptosis, excess cell division, and vasoconstriction predominate [161].

Certain neurohormones can increase production of reactive oxidative species (ROS) and reactive nitrogen species (RNS) via diverse mechanisms such as NAD(P)H oxidases, xanthine oxidase, cytochrome P450, and autooxidation of catecholamines [162]. Uncoupling of endothelial nitric oxide synthase (eNOS) can reduce available nitric oxide (NO) and increase superoxides, contributing to the imbalance between ROS and antioxidants - so called oxidative stress [163]. Oxidants can cause direct cellular damage via oxidation of molecules

such as lipids (lipid peroxidation) and DNA, and can promote contractile dysfunction by influencing enzymes involved in excitation-contraction coupling [149, 162]. They can also alter transcription factors such as nuclear factor- κ B (NF- κ B) or hypoxia-inducible factor-1 (HIF-1), with a range of downstream effects [162]. The milieu established by oxidative imbalance favours adverse myocardial remodelling with inactivation and induction of cell damage, inflammation, and apoptosis.

Relationship between RV afterload, myocardial coronary perfusion, and RV performance

Unique physiology dictates that RV pressurization impacts both myocardial coronary blood supply (reducing the pressure index, PI: difference between aortic and RV pressures throughout the cardiac cycle) and 'demand' (increasing wall tension which can be approximated by the tension time index, TTI: area under the RV pressure curve in systole). Autoregulation of the coronary bed and enhanced myocardial oxygen extraction may compensate but following maximal vasodilation, blood flow becomes solely dependent on forward pressure and, therefore, imminently susceptible to further perturbations in the supply:demand balance (e.g. exercise, tachycardia, progression of PVD). In this context, coronary blood supply may be insufficient to maintain energy-dependent cellular processes (particularly during physiologic stress such as exercise), leading to downregulation of contractility to restore the metabolic balance ('perfusion:contraction matching') [164]. It can be postulated therefore that RV ischemia may contribute to an impaired physiologic reserve and RV failure in PAH.

Early pre-clinical work first shed light on these important relationships by demonstrating: increasing RV AL caused subendocardial ischemia in the presence of a patent right coronary

artery (RCA); occlusion of the RCA had negligible impact on overall cardiac performance under control conditions, but promoted more significant RV dysfunction when AL was elevated; RV performance could be restored by increasing RV blood supply, even when AL remained high; and, the ratio between RCA driving pressure (pressure index (PI): area under RV systolic curve) and RV oxygen demand (tension time index (TTI): area under RV pressure curve during systole) decreased as RV AL was elevated in a step-wise fashion, and that at very high loads, hyperemic coronary flow reserve was disappeared [140, 165-167] [168]. Together, these studies suggest that right coronary flow is increasingly important as the RV becomes stressed in systole and RV ischemia may underlie the transition from RV adaptation to failure.

Considering the compelling pre-clinical findings, there is a surprising lack of clinical studies exploring relationships between RV AL, coronary status, and remodelling/performance, perhaps due to an inability to directly manipulate physiologic parameters to establish cause and effect. Van Wolferen *et al* assessed RCA and left anterior descending (LAD) flow using CMR in subjects with PAH and chronic thromboembolic pulmonary hypertension (CTEPH), comparing flow profiles with healthy controls [169]. In the LAD, flow profiles were similar between control and PH groups, whereas the systolic-to-diastolic flow ratio in the RCA was significantly lower in the PH group (0.39 ± 0.28 compared with 0.82 ± 0.43 , $P = 0.007$). Strong inverse correlations were found between RCA systolic-to-diastolic flow ratio and RV systolic pressure ($R = -0.83$ $P < 0.001$), and total mean coronary blood flow per gram myocardial tissue and RV hypertrophy ($R = -0.73$ $P < 0.001$), leading the authors to propose that RV systolic stress due to PH caused systolic compression of epicardial RCA flow.

Gomez *et al* observed scintigraphic evidence of subendocardial RV ischemia in a subset of patients with severe PAH. Presence of ischemia was associated with haemodynamic markers of RV dysfunction, including higher right ventricular end diastolic pressure (RVEDP; $p < 0.001$), higher right atrial pressure (RAP; $p < 0.037$) and lower mixed venous oxygen saturation ($p < 0.0001$) [170]. Biventricular myocardial perfusion reserve indexes (MPRI), calculated using adenosine perfusion CMR, were found to be lower in scleroderma-related PAH patients than scleroderma patients without PAH and healthy controls, suggesting impaired vasoreactivity or reduced coronary perfusion secondary to PAH in the scleroderma-PAH group [171]. This correlated closely with markers of RV workload and dysfunction, suggesting ischaemia may contribute to functional decline. Interestingly, LV MPRI was lower in the scleroderma PAH group than the scleroderma without PAH group, suggesting either the PAH itself, or more advanced scleroderma-related microvascular dysfunction, was the cause. Peripheral endothelial dysfunction has also been shown in PAH patients [172, 173], and has been correlated with pulmonary vasoreactive response to inhaled iloprost [173]. This raises the possibility that coronary microcirculatory dysfunction in PAH could contribute to lower MPRI, rather than haemodynamic alterations *per se* being the sole culprit (e.g. compression of coronary circulation in setting of increased wall stress, or reduced coronary driving pressure in the setting of systemic hypotension).

Calculating MPRI, particularly for the usually thin-walled RV, can be technically difficult, leading to interobserver variability [171]. Coronary sinus flow, which represents approximately 96% of total coronary efflux [174], can be measured via PC-CMR and incorporated with myocardial mass measurements to give myocardial blood flow per gram of myocardium [175]. Repeating these measurements with drug-induced hyperaemia permits calculation of coronary sinus flow reserve (CSFR), which has good correlation with the gold

standard measure of coronary flow reserve, positron emission tomography (PET) [176, 177]. Skrok *et al* found that mean CSFR was lower in PAH patients compared with healthy controls and the degree of impairment related directly to haemodynamic and CMR-derived measures of PAH severity and RV dysfunction, supporting a possible causative link between high RV AL, RV ischaemia, and RV dysfunction [178].

Clinical studies utilizing positron emission tomography (PET) have confirmed higher RV myocardial oxygen consumption (MVO₂) at rest in PAH patients compared with controls, which was attributed to increased RV power output (to overcome elevated AL) and RV mechanical inefficiency [179, 180]. A high RV oxygen extraction fraction (OEF) was noted in their iPAH cohort at rest (69% ± 17%, which is comparable to the accepted normal LV OEF of 60-80% and considerably higher than the expected normal RV OEF, estimated between 45-50%) [181, 182] [180, 183, 184], suggesting diminution of the OEF reserve and a greater dependence on perfusion for myocyte oxygen delivery. Not surprisingly therefore, Dubiel *et al* found that myocardial blood flow was higher (due to lower coronary vascular resistance) in the RV of patients with moderate PH (mPAP 31-60mmHg) [185]. Thus, even at rest, a moderately stressed RV has partially surrendered both OEF and perfusion reserves. Under severe stress (higher RV AL), Dubiel *et al* noted a failure to further augment resting blood flow (consistent with clinical studies utilising CMR-derived MPRI and CSFR), despite the commensurate rise in oxygen demand (greater RV power requirement). Therefore, during progression from moderate-severe PAH, there appears a disproportionate imbalance between increasing RV oxygen demand and declining OEF/perfusion reserves, raising the risk of myocyte hypoxia, particularly during physiologic stress. Moreover, this may be compounded by myocyte hypertrophy that is incompletely matched by neo-capillarization -

as has been observed in the RV of deceased, severe PAH patients – as flow per unit of myocardial mass decreases [153].

Measurement and manipulation of parameters that determine RV coronary status (supply and demand) may therefore be of considerable clinical use. In a canine model, Buckberg and associates showed that an index reflecting the ratio of LV myocardial oxygen supply (area between diastolic aortic and LV pressure, DPTI) and ‘demand’ (area under LV systolic pressure curve, SPTI) calculated from routine invasive parameters could predict subendocardial ischaemia [186]. This was confirmed in humans, with a DPTI/SPTI <0.45 predicting ischemic electrocardiogram (ECG) changes during strenuous exercise [187]. An analogous index of myocardial oxygen supply:demand for the RV was proposed by Cross [166]. Since the RV receives RCA flow during systole and diastole, the right coronary driving pressure or ‘supply’ (termed the pressure index, PI) was proposed as the area reflecting the aortic–RV pressure difference throughout the cardiac cycle while ‘demand’ (termed the tension time index, TTI) was estimated as the area under the RV systolic pressure curve. Using canine models, Fixler *et al* showed the PI/TTI decreased in proportion to the degree of RV pressure overload and that under severe stress, hyperaemic coronary flow reserve disappeared [168]. Later work showed preferential subendocardial ischemia in response to acute RV pressure loading, with a decrease in the endocardial:epicardial PI/TTI with increasing load [165, 188]. Finally, in an experimental pulmonic stenosis model of RV systolic hypertension, compensated RV function transitioned to RV failure in response to the opening of a systemic to pulmonary shunt which reduced PI and, therefore, RV coronary supply [189].

Despite these pre-clinical data and the attractive simplicity of the PI/TTI measurement, there are no apparent published reports exploring relationships between RV AL, PI/TTI, hyperaemic coronary flow reserve, and RV performance in humans. Furthermore, relationships between RV micro- and macro-vascular coronary status and cardiopulmonary reserve in the context of PVD/PAH are unclear. Since PVD is progressive despite contemporary treatments and lung transplantation is neither universally available nor suitable, directly targeting determinants of RV oxygen supply, demand, and utilization may provide additional treatment avenues, making the exploration of these relationships worthwhile.

1.3 SUMMARY

Pulmonary arterial hypertension is a rare clinical condition with an unacceptably poor prognosis despite the availability of effective but non-curative medical therapies. While first and foremost a pulmonary vasculopathy, characterized by obliterative remodelling at the proximal microcirculatory level, diagnosis is contingent upon sequelae that only arise once vascular lesions are fully developed and the pulmonary vascular bed is considerably compromised (that is, pre-capillary pulmonary hypertension). No validated method exists to detect pulmonary vascular disease during its initial stages, which may be an opportune time to initiate therapy. Exercise haemodynamic stress testing of the cardiopulmonary unit - with the intention of unmasking a depleted reserve - is pathophysiologically attractive, but limited by technical and methodological concerns which are compounded by non-invasive measurement techniques (e.g. Doppler echocardiography). Surrogate markers of pulmonary haemodynamics, accurate and reproducible indices of right ventricular structure and function, and proximal pulmonary vessel dynamics can all be reliably obtained without ionizing radiation during a single cardiac magnetic resonance imaging scan. Stressing the

cardiopulmonary using intravenous adenosine and monitoring changes in CMR-derived indices may afford an alternative, novel approach to detecting a depleted reserve. Following on, this may also provide a unified means to longitudinally monitor treatment response and disease progression in established PAH. Finally, pre-clinical and clinical studies suggest that an imbalance between RV myocardial oxygen supply and demand may contribute to RV pump failure, the primary cause of premature mortality in PAH, although our understanding of the relationships between severity of pulmonary vascular disease, RV coronary perfusion, RV remodelling and performance, and cardiopulmonary reserve in the clinical setting is limited.

CHAPTER 2

STUDY PROTOCOL

INTRODUCTION

The purpose of the present study is to design and assess feasibility, safety and tolerability of a non-invasive methodology to quantify pulmonary vasoreactive response in subjects with known or suspected PAH, and proceed to explore whether this methodology may afford a means to detect early pulmonary vascular disease and/or provide prognostic information (at baseline and longitudinally over time). Since this is a single-centre study employing a novel approach to the interrogation of the cardiopulmonary unit in the context of PVD, it is important to clearly outline and justify the protocol design (for the purposes of, for example, external validation). While methodology will also be covered in the results chapters (Chapters 3-5), this chapter will detail the CMR and RHC protocols in full, and rationalize the chosen intravenous adenosine infusion regimen.

STUDY OVERVIEW

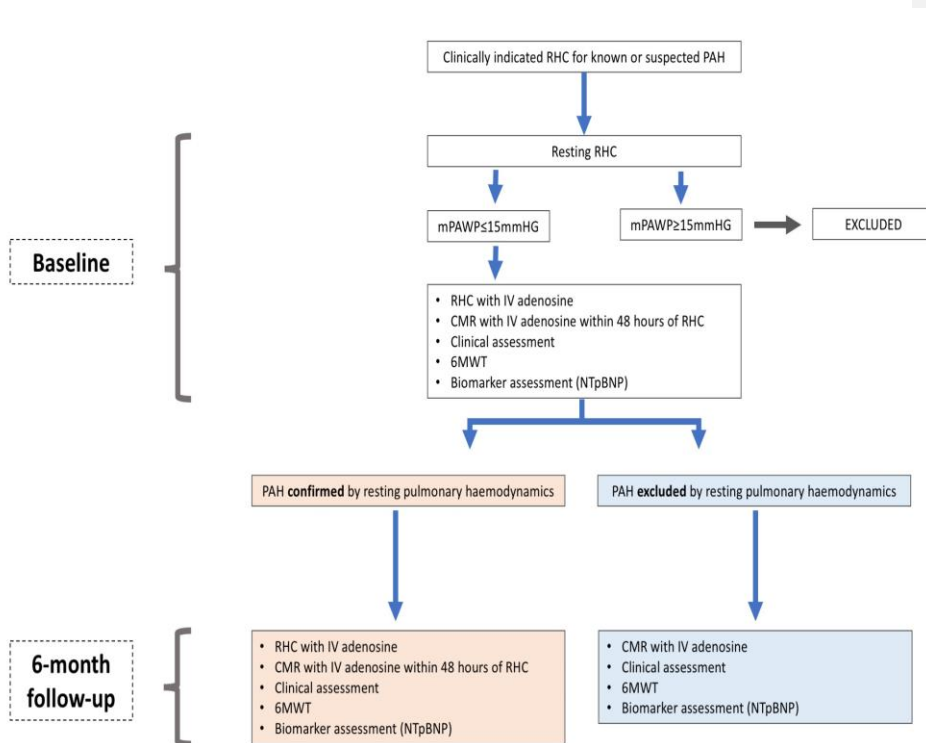


Figure 1: Study overview for participants with known or suspected PAH. Age and sex matched healthy volunteers will undergo a single CMR with intravenous adenosine using the same CMR protocol.

RIGHT HEART CATHETER PROTOCOL

1. Patient preparation:
 - a. No caffeine for 24 hours prior.
 - b. Clear fluids only for 4 hours prior.
 - c. Insert two 18-21 G peripheral intravenous cannulae (antecubital fossae).
2. In the catheterization laboratory:
 - a. No supplemental oxygen.
 - b. Sedation at the discretion of proceduralist (intravenous midazolam and/or fentanyl).
3. Right femoral venous access. 7 French sheath.
4. 7 French Swan-Ganz thermodilution pulmonary artery catheter (Edwards Lifesciences, California, USA).
5. Zero and fix transducer at level of mid-thorax.
6. Resting pulmonary haemodynamic assessment (waveforms to be recorded for offline analysis):
 - a. Right atrial pressure.
 - b. Right ventricular pressure.
 - c. Pulmonary artery pressure.
 - d. Pulmonary arterial wedge pressure: catheter tip in zone 3 lung regions.
Confirm by waveform visualization and oxygen saturation.
 - e. Cardiac output by thermodilution (triplicate).
7. Saturation run at discretion of proceduralist (recommended if a new case).

8. **If resting mPAWP <15mmHg**, proceed to vasoreactive challenge with intravenous adenosine:
 - a. Start at 70mcg/kg/min infusion via peripheral line.
 - i. After 2 minutes: Repeat measurement of pulmonary artery pressure, PAWP (do not confirm with SaO₂), CO by thermodilution in triplicate.
 - ii. Repeat arm-cuff systemic blood pressure.
 - b. Increase infusion rate to 140mcg/kg/min.
 - i. After 2 minutes: Repeat measurement of pulmonary artery pressure, PAWP (do not confirm with SaO₂), CO by thermodilution in triplicate.
 - ii. Repeat arm-cuff systemic blood pressure.
 - c. Increase infusion rate to 210mcg/kg/min.
 - i. After 2 minutes: Repeat measurement of pulmonary artery pressure, PAWP (do not confirm with SaO₂), CO by thermodilution in triplicate.
 - ii. Repeat arm-cuff systemic blood pressure.
 - d. Cease infusion.
9. Perform diagnostic coronary angiography via femoral or radial arterial access **IF** not performed in the preceding 3 years.
10. Recorded waveforms to be directly visualized offline to ensure uniform haemodynamic measurements:
 - a. Sinus rhythm: mean of 3 end-expiratory pressure measurements.
 - b. Atrial fibrillation: mean of 7 end-expiratory pressure measurements.
 - c. PAWP to be measured at the point corresponding to the *a* wave.

CARDIAC MAGNETIC RESONANCE IMAGING PROTOCOL

Cardiac magnetic resonance imaging will be performed with a 1.5 T magnet (Magnetom, Siemens, Erlangen, Germany) according to the protocol shown in Figure 2.

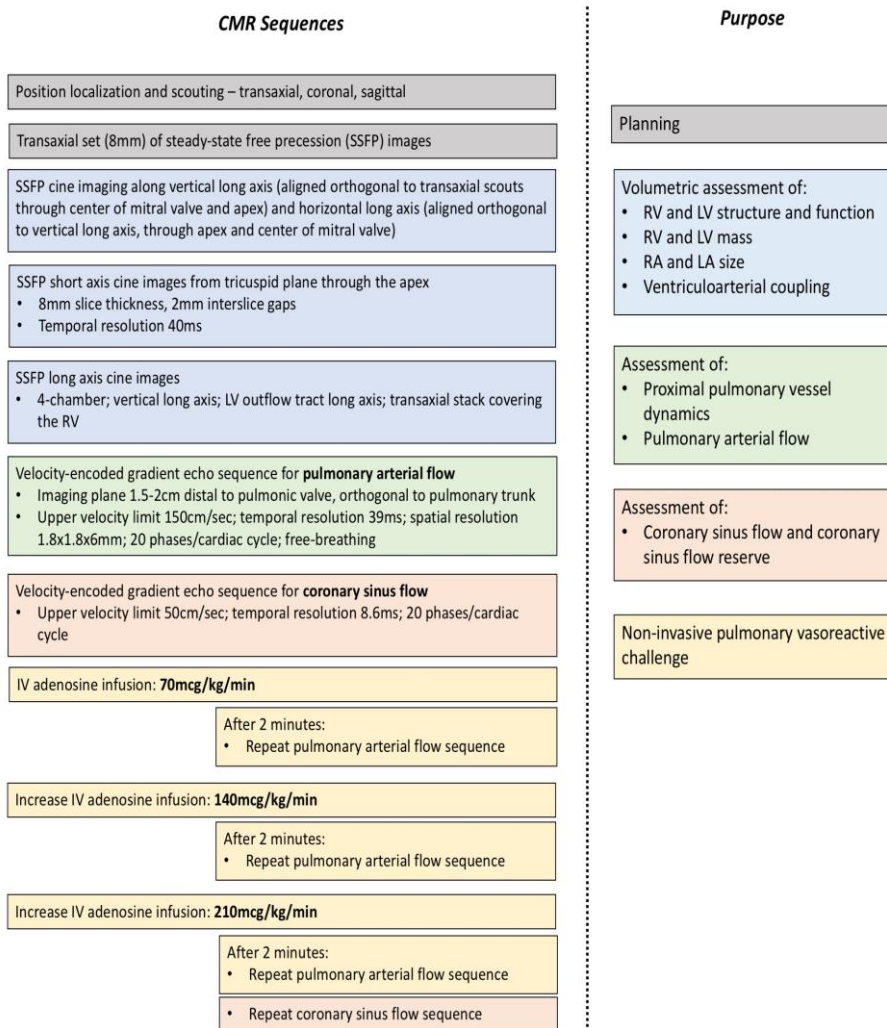


Figure 2: CMR protocol (left) and purpose of chosen sequences (right).

INTRAVENOUS ADENOSINE DOSING

The recommended regimen for intravenous adenosine infusion for invasive pulmonary vasodreactive testing is to start at 50mcg/kg/min and increase in 50mcg/kg/min increments every 2 minutes to a maximum dose of 350mcg/kg/min, unless limited earlier by side effects [40]. Two of the largest trials employing IV adenosine for this purpose postdate these guidelines. They found a dose range of 50mcg/kg/min-200mcg/kg/min adequate for assessment of vasoreactivity, with higher doses increasing side effects without additional benefit [113, 190]. Furthermore, the doses causing maximal reduction in mPAP were much lower than expected (88 ± 40 mcg/kg/min and 78 ± 23 mcg/kg/min), suggesting recommended doses are too high. In the coronary circulation, maximal hyperaemia may not be achieved at the commonly used dose of 140mcg/kg/min in a small proportion of subjects, but is very rare at a dose of 210mcg/kg/min, even in the setting of recent caffeine intake [191-193].

Intravenous adenosine can cause transient chest tightness, dyspnoea, palpitations, facial flushing, and mild reductions in systemic arterial pressure [194] which abate quickly upon cessation, given its short half-life (~10 seconds) [195]. Stimulation of adenosine receptors in the atrioventricular node and bronchial smooth muscle can promote bradycardia and bronchospasm respectively, which are rarely clinically significant unless there are predisposing co-morbidities. Overall tolerability and safety of adenosine perfusion CMR (for assessment of ventricular ischaemia) is acceptable at doses of 140mcg/kg/min and 210mcg/kg/min [191, 193]. Bortaso *et al* reported minor adverse events (flushing, chest discomfort, dyspnoea) in 27% of 362 adenosine perfusion CMR scans at a dose of 140mcg/kg/min, and no major adverse events [196]. Studies using higher doses (350mcg/kg/min – 500mcg/kg/min) for vasoreactivity testing in PAH patients reported higher

rates of minor adverse events (flushing, chest discomfort, dyspnoea) [92, 93, 113, 190], but major adverse events remained rare. A significant decrease (> 20%) in systemic arterial pressure has been reported in 2 of 104 patients in one study [113], and less frequently elsewhere [92, 93, 190, 197].

Rationale for the dosing regimen of the present study (70mcg/kg/min, increasing in 70mcg/kg/min increments every 3-4 minutes to a maximum dose of 210mcg/kg/min, unless limited earlier by side effects) is:

- Permit dose-response relationships to be interrogated;
- Ensure maximal hyperaemia is achieved (maintain sensitivity to detect a 'clinically meaningful' response); and,
- Minimize risk of minor and major adverse events.

CHAPTER 3

NON-INVASIVE ASSESSMENT OF CARDIOPULMONARY RESERVE: TOWARD EARLY DETECTION OF PULMONARY VASCULAR DISEASE

KEY WORDS:

- Magnetic resonance imaging
- Right ventricle
- Hypertension, pulmonary
- Haemodynamics
- Adenosine

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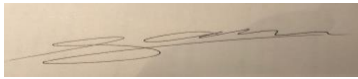
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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

- iv. the candidate's stated contribution to the publication is accurate (as detailed above);
- v. permission is granted for the candidate to include the publication in the thesis; and
- vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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ABSTRACT

Background

Pulmonary arterial hypertension (PAH) represents a late stage of progressive microcirculatory remodelling, or ‘pulmonary vascular disease’ (PVD). Earlier detection of PVD should improve prognosis with current therapies, although validated, non-invasive methods to do so remain elusive. We postulated that changes in pulmonary arterial blood flow velocity or pulsatility in response to standardised intravenous (IV) adenosine infusion, measured using cardiac magnetic resonance imaging (CMR), would provide a novel and non-invasive means to measure ‘cardiopulmonary reserve’, providing proof-of-concept for the detection of pre-clinical PVD.

Methods

Patients with known or suspected PAH and a clinical indication for a right heart catheter (RHC) and matched healthy volunteers were studied. Exclusion criteria were <18 years old, pregnancy, a high cardiac output (CO) state, mean pulmonary arterial wedge pressure (mPAWP) > 15mmHg, or a contraindication to CMR or adenosine.

RHC (invasive arm) and CMR (non-invasive arm) were performed within 48 hours.

Measurements (**RHC**: mean pulmonary arterial pressure [mPAP], mean pulmonary arterial wedge pressure [mPAWP], CO by thermodilution, and pulmonary vascular resistance [PVR];

CMR: phase-contrast imaging through the pulmonary trunk to calculate average pulmonary blood flow velocity [meanPAvel] and pulsatility) were taken at rest and during intravenous adenosine infusion (after 2 minutes at each dose: 70-, 140-, and 210mcg/kg/min). PAH was confirmed (PAH group, n=17) or excluded (High Risk group, n=9) using standard resting haemodynamic criteria. Age and sex matched healthy volunteers underwent CMR only (Control group, n=10).

Results

Invasively, the High Risk group had greater dose-dependent haemodynamic changes with adenosine: PVR reduction 18%±8% greater ($P<0.05$) due predominantly to CO augmentation (27%±13% greater, $P<0.05$) rather than transpulmonary gradient reduction (TPG = mPAP–mPAWP; 5.7%±9% greater, $P=0.57$). Correlation between CMR-derived meanPAvel and haemodynamic measurements (positive with CI, negative with mPAP /PVRI) was strong at rest and throughout adenosine infusion ($R^2>0.32$, $P<0.05$ for all) whereas correlation between CMR-derived pulsatility and haemodynamic measurements weakened during adenosine. Intra- and interobserver variability was greater for pulsatility compared with meanPAvel.

Differences between group average meanPAvels were smallest at rest (9.7±2.2cm/s vs. 14.9±2.7cm/s vs. 18±2.5cm/s, PAH vs. High Risk, $P<0.0001$; HR vs. Control, $P=0.02$) and largest at peak hyperaemia (11.9±3cm/s vs. 22±3.4cm/s vs. 33.7±4.4cm/s, $P<0.0001$ between all) reflecting dose-dependent changes of +27±24%, +48±29% and +86±26% for PAH, High Risk and Healthy Control groups respectively ($P=0.052$ for PAH vs. High Risk; $P=0.01$ for High Risk vs. Healthy Controls). Receiver operator characteristic analyses revealed excellent capacity for meanPAvel at hyperaemia to differentiate between groups (PAH vs. High Risk: AUC=0.99; High Risk vs. Controls: AUC 1.0). No dose-dependent relationship

were identified between haemodynamic changes and changes in pulsatility, and diagnostic performance of pulsatility was inferior to that of meanPAvel.

Conclusion

CMR-derived meanPAvel is safe and feasible surrogate measure of pulmonary haemodynamic changes during standardized adenosine infusion, with meanPAvel at hyperaemia performing excellently as a functional correlate for cardiopulmonary reserve across a spectrum of clinical risk phenotypes. This may provide a means to detect pre-clinical PVD, although larger studies are necessary to validate these findings.

BACKGROUND

Pulmonary arterial hypertension (PAH) is a progressive disease characterised by a pathological increase in the resistance of the pulmonary circulation, predominantly due to obliterative remodelling of the microvasculature ('pulmonary vascular disease', PVD). Owing to abundant reserve, symptoms and haemodynamic abnormalities remain absent until most of the functional microcirculation has been irreversibly damaged, contributing to the poor prognosis that persists despite recent therapeutic advances. Earlier disease detection should confer prognostic advantage using available disease-modifying therapies, a notion supported by registry data [145]. However, current screening of high-risk patient groups remains reliant on the non-invasive identification of deranged pulmonary haemodynamics or their impact on the right ventricle (RV), rather than targeting the impaired physiologic reserve that undoubtedly precedes these findings.

Presently, there is no validated method to detect PVD before measureable haemodynamic changes arise ('early' PVD). Borderline pulmonary hypertension (resting mean pulmonary arterial pressure (mPAP) of 21-24mmHg) has been associated with higher PAH incidence in scleroderma patients [198], but further research is required to clarify the natural history in a more diverse patient group. Exercise induced pulmonary hypertension is backed by sound pathophysiological rationale and may suggest a pre-clinical stage of the disease, although the exact definition and implications of a 'pulmonary hypertensive response' remain to be elucidated [199] [14, 48]. Furthermore, while a non-invasive method is preferable, acquisition of data by echocardiography can be technically demanding and susceptible to imprecision [200, 201]. In PAH patients, a greater response to pulmonary vasodilators at the time of diagnostic right heart catheter (RHC) has been associated with an improved prognosis

across a range of subtypes [97-100, 202], and while a definitive explanation remains elusive, this may in part relate to greater reserve. To date there are no published reports of non-invasive pulmonary vasoreactivity tests.

We hypothesised that a standardised non-invasive pulmonary vasoreactive protocol utilising intravenous (IV) adenosine and cardiac magnetic resonance imaging (CMR) would permit quantification of pulmonary vasoreactivity, so that subjects with confirmed PAH, suspected but excluded PAH (high risk for incident disease) and healthy controls would display discrepant responses. If observed, this tool may enable the detection of PVD across a spectrum of patients with clinical and pre-clinical pathology.

MATERIALS AND METHODS

Study Population

Subjects with known or suspected PAH and a clinical indication for a right heart catheter (RHC) were recruited through a single tertiary-referral centre. Exclusion criteria were age <18 years old, pregnancy, known or suspected PAH due to congenital heart disease with systemic-to-pulmonary shunt or portopulmonary hypertension, or a contraindication to CMR or IV adenosine. Broad inclusion criteria were applied to reflect a contemporary referral population and to permit recruitment of participants across a spectrum of PVD severity. Right heart catheterization for suspected PAH was deemed clinically indicated by pulmonary hypertension experts following a comprehensive review of clinical status and ancillary investigations, in accordance with guidelines [40].

Participants underwent RHC and PAH was confirmed or excluded by standard haemodynamic criteria (mean pulmonary arterial pressure, mPAP, >25mmHg; mean pulmonary arterial wedge pressure, mPAWP, <15mmHg; and pulmonary vascular resistance, PVR >3 Woods units, WU). All participants with a mPAWP <15mmHg underwent vasoreactive testing with IV adenosine, and a CMR scan within 48 hours. Participants with mPAWP > 15mmHg were excluded and did not undergo vasoreactive testing. Age and sex matched volunteers were recruited and underwent the CMR component only. All participants provided written informed consent and protocols were approved by the local Research Ethics Committee.

RHC

Participants avoided caffeine for 48 hours prior. Right heart catheterisation was performed under light sedation and without supplemental oxygen via the right femoral vein, using a 7 F Swan-Ganz thermodilution pulmonary artery catheter (Edwards Lifesciences, California, USA). Comprehensive haemodynamic assessment was undertaken in accordance with guidelines [25]. Systemic arterial pressures were monitored via direct arterial measurement with a second fluid-filled pressure transducer, or arm cuff if arterial access was unavailable. A blood sample was taken for the assessment of N-terminal pro brain natriuretic peptide (NTpBNP) levels and centrifuged within 1 hour at 2800rpm for 10 minutes, and stored at -80°C until analysis using a commercially available ELISA kit (USCN Life Science, Texas, USA).

Intravenous adenosine (Adenoscan, 3mg/mL, Astellas Pharma, Tokyo, Japan) was administered via peripheral venous access (antecubital fossa) as a continuous infusion at three increasing doses: 70-, 140-, and 210mcg/kg/min. Haemodynamic assessment (PAP,

mPAWP, cardiac output [CO] in triplicate) was repeated after a minimum of two-minutes at each dose.

CMR

Cardiac MR imaging was performed using a 1.5 Tesla magnet (Magnetom, Siemens, Erlangen, Germany). Cine images of the atria and ventricles were acquired by steady-state free precession (SSFP) sequences, and were reconstructed into 25 cardiac phases to allow volumetric and functional analyses. Flow imaging was performed perpendicular to the main pulmonary artery during free-breathing, approximately 1.5-2cm above the level of the pulmonic valve, using a velocity-encoded gradient echo sequence with an upper velocity limit of 150cm/sec, temporal resolution 39ms, spatial resolution 1.8x1.8x6mm, 20 phases/cardiac cycle (Figure 1). Non-invasive pulmonary vasoreactivity testing was conducted using the same protocol as during RHC. Systemic blood pressure, arterial oxygen saturation, and heart rate (HR) were all non-invasively monitored during the infusion. Flow imaging was repeated after a minimum of 2-minutes infusion at each dose.

Images were analysed offline using specialised software (CMR42, Circle Cardiovascular Imaging Inc., Calgary, Canada). Biventricular endocardial and epicardial contours were manually drawn at end-diastole and end-systole. Right ventricular trabeculations and left ventricular papillary muscles were excluded from the blood pool. Atrial volumes were measured at end-systole. Main PA blood flow was measured by manually outlining the inner perimeter of the vessel at all 20 reconstructed cardiac phases, reflecting an entire cardiac cycle. The key parameters of interest during adenosine infusion were the mean pulmonary arterial blood flow velocity across the cardiac cycle (meanPAvel, Figure 1) and PA pulsatility ($[\text{maximum PA area} - \text{minimum PA area}] / \text{maximum PA area} * 100$).

Statistical Analysis

Categorical values are expressed as percentage, and normally distributed continuous variables expressed as means \pm standard deviations. Continuous variables were compared using Student t test or one-way analysis of variance (ANOVA). Scatterplots were visualized and Pearson correlation coefficients used to explore strength of linear relationships between meanPAvel/pulsatility and invasive haemodynamic parameters, and Spearman correlation coefficients used to explore strength of curvilinear relationships where there was a departure from Gaussian distribution. Receiver operating characteristic curve analysis investigated non-invasive detection of participant groups by CMR parameters, with areas under the curve compared using the method described by Hanley and McNeil [203]. Intra- and inter-observer variability was assessed using Bland-Altman analysis. A P value of <0.05 was considered statistically significant. Statistical analyses were performed using a software package (GraphPad Prism version 6.0, San Diego, California, USA).

RESULTS

Study Population

Forty-one participants were recruited (known or suspected PAH $n=31$, healthy volunteers $n=10$) and 5 excluded (mPAWP > 15 mmHg $n=4$; claustrophobia $n=1$). PAH was confirmed in 17 participants (PAH group: incident PAH $n=8$, prevalent PAH $n=9$) and excluded in 9 participants. For the purpose of this study, these participants were labelled 'high risk' for incident PAH (possible early PVD, 'High Risk' group) because of unexplained breathlessness ($n=9$), high prevalence of connective tissue disease (scleroderma $n = 5$; systemic lupus erythematosus $n = 1$), disproportionately reduced pulmonary diffusing

capacity for carbon monoxide, elevated NT-pro brain natriuretic peptide levels without myocardial or renal dysfunction, and 'borderline' abnormal resting haemodynamics (resting mPAP 21-24mmHg, n = 2). Demographic and clinical characteristics are presented in Table 1.

Safety and tolerability

Side-effects with adenosine were similar between groups. Facial flushing (84%), dyspnoea (81%), chest tightness (76%), and mild nausea (43%) were most common. Infusion was ceased prior to protocol completion in 2 participants (1 due to temporary, self-limiting sinus bradycardia; 1 due to severe nausea). There were no major adverse events.

Invasive pulmonary haemodynamics ('invasive arm')

Rest

Compared with the High Risk group, pulmonary pressures (diastolic, systolic, mean) and PVR were significantly higher in the PAH group, but there no significant between-group differences in RAP or CI at rest. Resting pulmonary haemodynamics were similar between 'incident' and 'known' PAH participants. Mean PAP was outside the accepted normal range in 4 High Risk participants (between 21-24mmHg) [42].

Adenosine stress

Greater dose-dependent haemodynamic changes were observed in response to IV adenosine in the High Risk group compared with the PAH group (Figure 2). Pulmonary vascular resistance decreased by $47\% \pm 23\%$ vs. $29\% \pm 16\%$ respectively (difference of $18\% \pm 8\%$, $P < 0.05$), due predominantly to greater augmentation of CI ($+59\% \pm 30\%$ vs. $+32\% \pm 30\%$;

difference of $27\% \pm 13\%$, $P < 0.05$) rather than decline in transpulmonary gradient (TPG = mPAP - mPAWP; $-9\% \pm 18\%$ vs. $-15\% \pm 30\%$, $P = 0.57$). Stroke volume index (SVI) and HR increased in both groups to drive CI augmentation, although HR increased significantly more in the No PAH group ($28\% \pm 7\%$ greater, $P < 0.05$). A small but statistically significant rise in mPAWP ($+2.6 \pm 2.7$ mmHg) was observed in the High Risk group although the mean value during maximal hyperaemia remained < 15 mmHg (13 ± 2 mmHg at 210 mcg/kg/min). There were no significant changes in systemic blood pressure in either group. A summary of these findings is provided in Table 2.

Cardiac magnetic resonance imaging ('non-invasive arm')

Rest

Volumetric CMR parameters revealed significantly lower RVEF, higher RV mass index, and greater indexed RA volume in the PAH group compared with High Risk and Healthy Control groups, but no significant differences between High Risk and Healthy Control groups.

MeanPAvel was the only parameter to vary significantly between all groups (9.7 ± 2.2 cm/s vs. 14.9 ± 2.7 cm/s vs. 18 ± 2.5 cm/s for PAH, High Risk and Healthy Control groups respectively; PAH vs. High Risk and High Risk vs. Healthy Control $P < 0.05$). Pulmonary artery pulsatility was significantly lower in the PAH group than both other groups, but there was no significant difference between High Risk and Healthy Control groups ($20 \pm 9\%$ vs. $43 \pm 14\%$ vs. $49 \pm 14\%$ respectively; PAH vs. High Risk $P < 0.05$; High Risk vs. Healthy Control $P = \text{NS}$). Left ventricular myocardial mass, left atrial volume, and LVEF were similar between all groups. These parameters are presented in Table 2.

Adenosine stress

A linear, dose-dependent increase in meanPAvel was observed in all groups during adenosine stress (Figure 3 and Supplement Table 3). The magnitude of increase was significantly greater in the High Risk group compared with the PAH group (meanPAvel at rest 14.9 ± 2.7 cm/s vs. 9.7 ± 2.2 cm/s respectively and meanPAvel at maximal hyperaemia 21.7 ± 3.4 cm/s vs. 11.9 ± 3.3 cm/s respectively; 1/linear regression slope = 90 vs. 30 respectively; $P < 0.0001$ for all), and significantly greater in the Healthy Control group compared with both other groups (meanPAvel at rest 18 ± 2.5 cm/s and meanPAvel at maximal hyperaemia 33.7 ± 4.3 cm/s; 1/linear regression slope = 13; $P < 0.0001$ compared to other groups). No clear dose-response relationship in PA pulsatility was observed and there were no significant between-group differences in the relative change in pulsatility between rest and maximal hyperaemia (Table 2). Intra-observer and inter-observer variability was small for meanPAvel but there was consistent bias and broad limits of agreement for pulsatility (Table 4).

Correlation between CMR flow-imaging parameters and invasive haemodynamics

Rest

Strong, inverse, curvilinear relationships were found between meanPAvel with PVR ($R = -0.86$, $P < 0.0001$) and mPAP ($R = -0.76$, $P < 0.0001$), and a moderate, positive, linear relationship observed between meanPAvel and CI ($R = 0.66$, $P < 0.0001$). There was no correlation between meanPAvel and mPAWP ($R = -0.05$, $P = \text{NS}$). Moderate-strong correlation between pulsatility and haemodynamic parameters was also observed with similar inverse, curvilinear relationships with PVR ($R = -0.74$, $P < 0.0001$) and mPAP ($R = -0.69$,

P<0.0001) and positive linear relationship with CI (R=0.65, P<0.0001). Correlation coefficients are presented in Table 3 and scatterplots in Figure 4.

Adenosine stress

Correlation remained strong during adenosine infusion between meanPAvel and haemodynamic parameters, but weakened between pulsatility and haemodynamic parameters. (Table 3).

Non-invasive discrimination between groups

PAH vs. High Risk

At rest, meanPAvel (area under the ROC curve, AUC: 0.98, CI 0.94 – 1.02) and pulsatility (AUC: 0.95, CI 0.88 – 1.01) showed excellent diagnostic performance and were unchanged when only 'incident' PAH participants were analysed. There was a non-significant improvement in the AUC for meanPAvel at peak hyperaemia (AUC: 0.99, CI 0.96 – 1.01, P=NS vs. meanPAvel at rest), and a non-significant decrease in the AUC for pulsatility at peak hyperaemia (AUC: 0.86, CI 0.72 – 1.00, P=NS vs. pulsatility at rest).

High Risk vs. Healthy Controls

At rest, meanPAvel demonstrated good diagnostic capacity (0.81, CI 0.61 – 1.01) whereas diagnostic performance with pulsatility was poor (AUC 0.63, CI 0.36 – 0.90). Diagnostic performance of meanPAvel at peak hyperaemia was excellent and significantly better than meanPAvel at rest (AUC 1.0, CI 1.0 – 1.0, P = 0.03 vs. meanPAvel at rest). No significant change in the AUC was found for pulsatility at peak hyperaemia (AUC 0.57, CI 0.29 – 0.84, P=NS vs. pulsatility at rest).

DISCUSSION

Adenosine, administered intravenously, promotes preferential pulmonary over systemic vasodilation via endothelial-independent mechanisms at the proximal microcirculatory level where PVD predominates, causing CO augmentation without direct inotropic effect [91, 93, 113] [87, 92]. Haemodynamic changes during IV adenosine therefore reflect microcirculatory dilatation/recruitment, the capacity of the RV to augment flow in response to afterload reduction, or a combination thereof. 'Microcirculatory reserve' is likely to govern the magnitude of haemodynamic response early in the disease course whereas in more severe PAH, 'RV reserve' may also contribute (inotropic and/or chronotropic). Both are important and intricately linked, hence the term 'cardiopulmonary reserve'.

Invasively, average haemodynamic changes in response to adenosine in the High Risk group exceeded those in the PAH group, confirming greater cardiopulmonary reserve. Previous studies have demonstrated a prognostic advantage associated with greater haemodynamic improvement during vasoreactivity testing in established PAH patients not meeting traditional cut-offs for a 'true' vasoreactive response (i.e. reduction in mPAP by ≥ 10 mmHg to a mPAP ≤ 40 mmHg with preserved or increased CO), suggesting a relatively greater vasoreactive response may reflect a phenotype with more vasoconstriction, less vascular remodelling and, hence, greater vascular reserve [96] [97] [98] [99] [100] [202] [7]. The greater PVR reduction with adenosine in the High Risk group was driven primarily by greater CO augmentation which, in turn, was driven by a greater rise in heart rate. It is unclear whether this reflected an appropriate physiologic response to discrepant degrees of pulmonary microvascular recruitment, or whether autonomic dysfunction in the PAH group

may have played a role. Autonomic dysfunction is described in PAH and associated with exercise intolerance and ventilatory inefficiency [204, 205] [206], and a failure to increase HR during exercise has been linked with poor survival [207] [208].

In agreement with a pre-clinical study [83], strong correlation between CMR-derived meanPAvel and haemodynamic parameters was found throughout adenosine-stress, suggesting this may be an appropriate clinical surrogate marker of haemodynamic changes during vasoreactive testing. Since meanPAvel is thought to slow due to microvascular obstruction (PVD), PA dilation and RV systolic dysfunction [82], it is unsurprising that significant between-group differences in meanPAvel were observed at rest. Importantly, between-group differences increased during adenosine-stress and were greatest during maximal hyperaemia, suggesting meanPAvel at peak hyperaemia performs as an excellent functional correlate for cardiopulmonary reserve across a spectrum of clinical risk phenotypes, as determined by ‘traditional’ haemodynamic (rest and vasoreactive response), clinical and biomarker characteristics. The non-linear, inverse relationship between meanPAvel and PVR (Figure 4) makes meanPAvel at peak hyperaemia a compelling non-invasive parameter to apply to the detection of ‘early’ PVD since early in the disease course (low PVR), any adenosine-induced PVR reduction will be magnified by a relatively greater meanPAvel increase, as illustrated by the divergence of High Risk and Healthy control groups in the present study. Finally, we postulate that the superior diagnostic performance of meanPAvel at peak hyperaemia over the relative change in meanPAvel may be due to the impact of ventilation-perfusion matching, hypoxic vasoconstriction or anxiety at rest, which are ameliorated at peak hyperaemia.

Reduced global arterial compliance and increased local PA stiffness are recognized early changes in PAH and have been shown to be predict mortality, response to vasoreactivity testing, and functional capacity as determined by 6MWD [58, 60, 63], and may directly contribute to disease progression by promoting downstream vascular remodelling [63] [60] [69]. In the present study, pulsatility was significantly lower at rest in the PAH group but no significant difference between High Risk and Control groups was found. Since RV pulsatile afterload is known to vary inversely with the resistive afterload in a non-linear fashion, we hypothesized that pulsatility would increase during adenosine-stress in proportion to PVR reduction, but no dose-dependent change in pulsatility was observed during adenosine-stress. This may be due to susceptibility of this parameter to small changes in imaging plane and manual measurement (as suggested by intra- and inter-observer variability), or haemodynamic changes induced by adenosine may alter the resistance-compliance relationship.

Study limitations

Key limitations of this study are the small sample size, single-centre design, and lack of clinical outcome measures. The small sample size and large number of measured parameters which exhibit co-linearity precludes multivariate analyses. Participants with suspected but excluded PAH were labelled high risk for incident PAH (possible pre-clinical PVD), which can only be validated by long-term follow-up. The dosing regimen for this study was proposed to standardise exposure, achieve hyperaemia, and ensure clinically meaningful responses would not be missed, although it is possible that other doses may have been more appropriate. Our findings should therefore be considered hypothesis generating until larger trials with long term clinical follow-up are conducted.

CONCLUSION

This study demonstrates safety, feasibility and proof-of-concept for a standardized, novel, non-invasive method to assess cardiopulmonary reserve as it pertains to PVD and its sequelae, PAH, using CMR-derived measurement of mean pulmonary arterial blood flow velocity during adenosine-induced hyperaemia. Sound rationale, non-invasive acquisition, ease of measurement, and robust inter- and intra-observer variability should facilitate larger validation trials. If confirmed, such a tool should afford the earliest possible detection of PVD, which may prove an attractive time point for therapy.

FIGURES

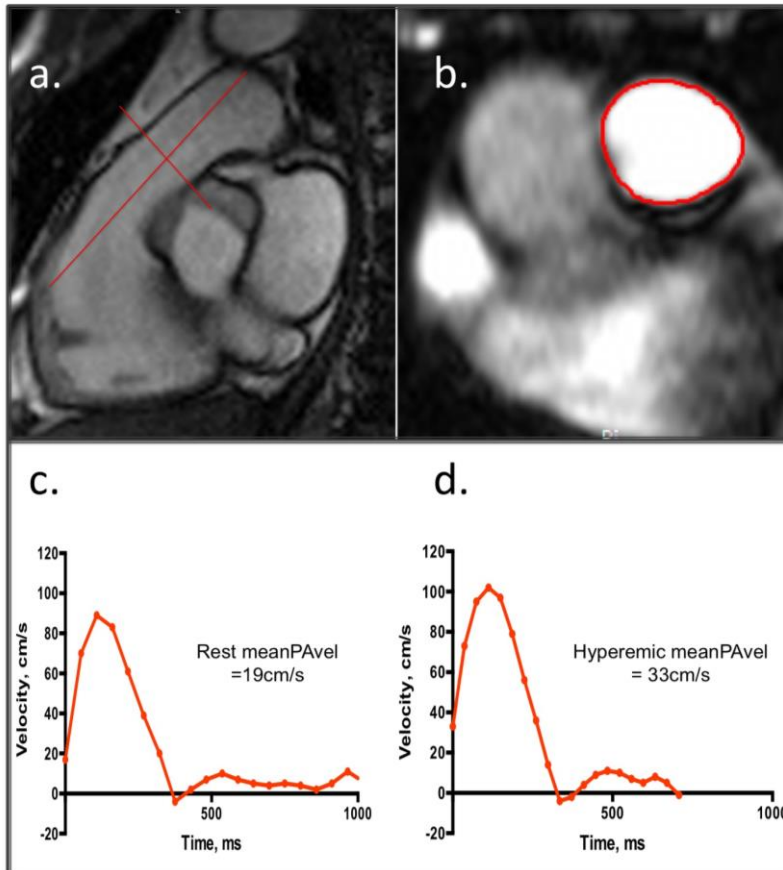


Figure 1: Reference sequences for flow imaging were two double-oblique orthogonal views acquired by SSFP along the main axis of the pulmonary trunk (a.). Flow imaging was performed perpendicular to the pulmonary trunk (b.) and repeated at all doses of adenosine infusion, allowing measurement of flow and magnitude related variables. A typical flow velocity profile is shown at rest (c.) and during hyperaemia (d.). Mean PA velocity was calculated by averaging blood flow velocity at all 20 reconstructed cardiac phases.

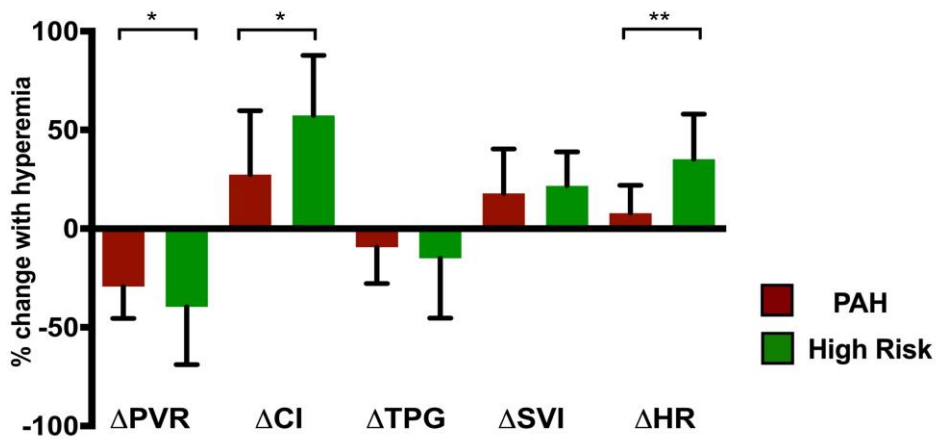


Figure 2: Haemodynamic changes at peak hyperaemia compared with rest.

ΔPVR = change pulmonary vascular resistance, ΔCI = change cardiac index, ΔTPG = change transpulmonary gradient (mPAP-mPAWP), ΔSVI = change stroke volume index, ΔHR = change heart rate.

* $P < 0.05$, ** $P < 0.005$,

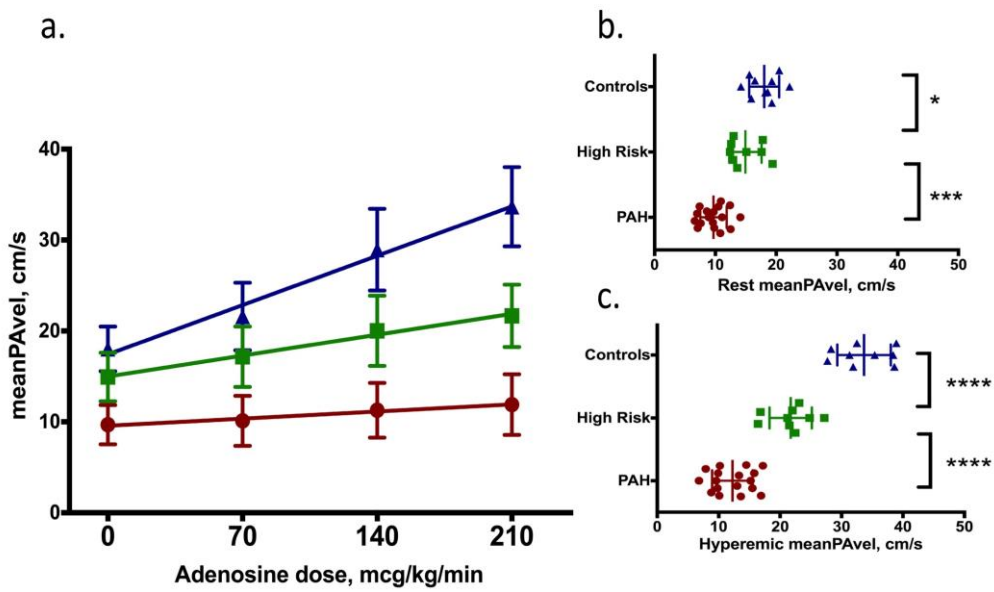


Figure 3: Change in meanPAvel during adenosine infusion (a.). Between-group differences were smallest at rest (b.) and greatest at peak hyperaemia (c.).

* P<0.05, **P<0.005, *** P< 0.0005, **** P<0.0001.

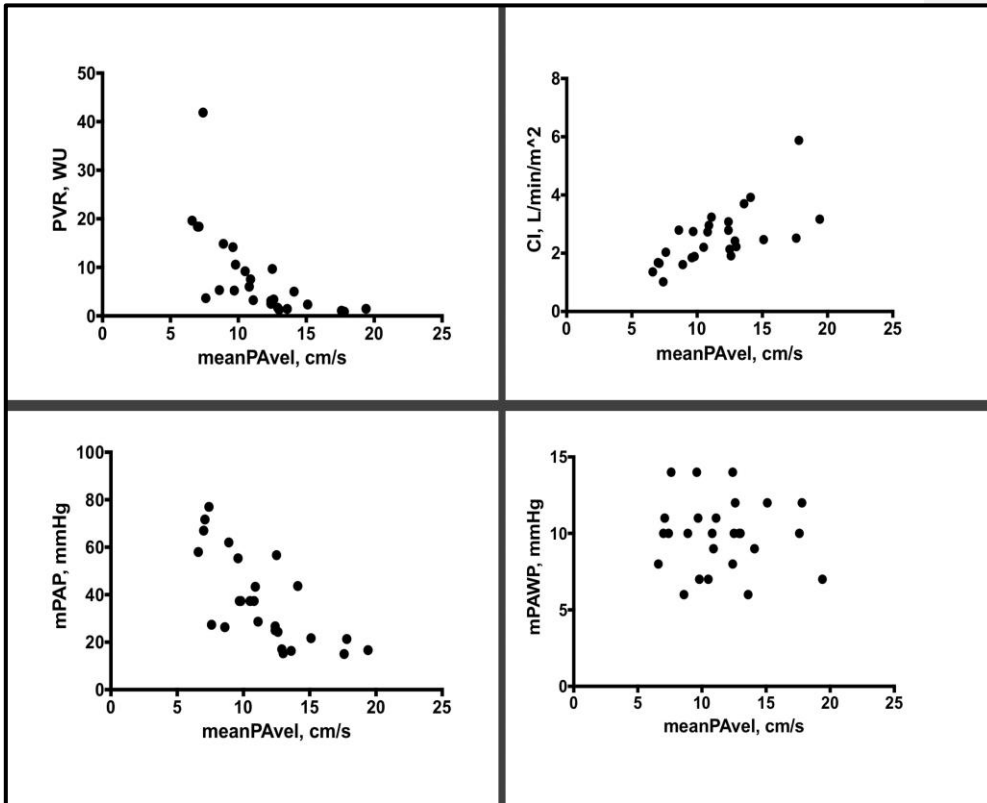


Figure 4: Scatterplots illustrating the relationship between meanPAvel and invasive haemodynamic parameters measured at rest.

PVR = pulmonary vascular resistance, CI – cardiac index, mPAP = mean pulmonary arterial pressure, mPAWP = mean pulmonary arterial wedge pressure, WU = Wood units.

TABLES

	PAH	High Risk	Healthy Controls	P value
Participants, n	17	9	10	
Female (%)	13 (76)	7 (78)	8 (80)	0.98
Age, years	54.3±14	60.2±14	46.9±12	0.13
Height, cm	162.6±12	164.1±9	165.6±7	0.75
Weight, kg	73.7±19	78±14	70.3±11	0.58
BSA, m ²	1.78±0.25	1.84±0.14	1.78±0.16	0.75
PAH aetiology (%)				
Idiopathic	12 (71)			
CTD-associated	5 (29)			
Comorbid conditions (%)				
Diabetes mellitus	4 (24)	0 (0)		<0.05
Smoking	2 (12)	0 (0)		<0.05
CTD	5 (29)	6 (67)		<0.05
NYHA functional class (%)				
II	5 (29)	9 (100)		<0.05
III	12 (71)	0 (0)		<0.05
Therapy (%)				
Endothelin receptor antagonist	1 (11)			
Phosphodiesterase-5 inhibitor	1 (11)			
Combination	7 (78)			
6MWD, m	400±104	480±59		0.07
NTpBNP, pg/ml	2203±1350	1555±824		0.29
Normalised, NTpBNP	14.1±9.6	7.7±4.8		0.12
Creatinine, µmol/L	81±21	75±34		0.58
D _L CO, %	52±17	61±15		0.3
%FVC/%D _L CO	1.6±0.88	1.4±0.18		0.48

Table 1. Demographic and clinical characteristics of participants.

BSA = body surface area, CTD = connective tissue disease, 6MWD = 6-minute walk distance, NTpBNP = N-terminal pro-brain natriuretic peptide, D_{LCO} = diffusing capacity for carbon monoxide; FVC = forced vital capacity.

	PAH			High Risk			Healthy Control		
	Rest	Hyper	Change (%)	Rest	Hyper	Change (%)	Rest	Hyper	Change (%)
Systolic SBP (mmHg)	128 ±20	132 ±23	4 ±9	135 ±14	142 ±17	6 ±6	132 ±8	135 ±9	3 ±7
Diastolic SBP (mmHg)	76 ±11	75 ±12	1 ±14	82 ±10	81 ±12	0 ±19	78 ±6	81 ±6	3 ±8
Heart Rate (bpm)	78 ±16	86 ±20	12 ±23	71 ±13	93 ±13	33 ±17*	64 ±7	99 ±19	54 ±20 [†]
PVR (WU)	12 ±10	10 ±10	-29 ±16	2 ±0.9**	1 ±0.5*	-47 ±23*			
mPAP (mmHg)	47 ±17	45 ±16	-7 ±11	19 ±4****	20 ±3****	+5 ±14			
CI (L/min/ m²)	2.3 ±0.8	2.9 ±1.2	+32 ±30	3 ±1.2	4.7 ±1**	+59 ±30*			
mPAWP (mmHg)	10 ±2	11 ±4	+22 ±35	10 ±3	13 ±2	+31 ±30			
RAP (mmHg)	8 ±5			5 ±3					
meanPAvel (cm/s)	10 ±2	12 ±3	+27 ±24	15 ±3*	22 ±3****	+48 ±29	18 ±3 [†]	34 ±4 ^{††††}	+86 ±26 ^{††††}
Pulsatility (%)	20 ±9	23 ±10	32 ±74	43 ±14*	37 ±10	-9 ±34	49 ±14 [†]	34 ±7	-25 ±25
RVEF (%)	40 ±18			61 ±8*			63 ±6 [†]		
LVEF (%)	66 ±10			69 ±3			67 ±4		
RVmassI (g/m²)	45 ±19			28 ±10*			24 ±6 [†]		

RAvolI (ml/m²)	85 ±38		50 ±22*		47 ±8 [†]	
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Table 2: RHC and CMR-derived parameters at rest and during adenosine-induced hyperaemia (Hyper).

SBP = systemic blood pressure; PVR = pulmonary vascular resistance; mPAP = mean pulmonary arterial pressure; CI = cardiac index; mPAWP = mean pulmonary arterial wedge pressure; RAP = right atrial pressure; meanPAvel = mean pulmonary arterial blood flow velocity; RVEF = RV ejection fraction; LVEF = left ventricular ejection fraction; RVmassI = indexed RV mass; RAvolI = indexed RA volume.

*P<0.05, **P<0.005, ***P<0.0001 for High Risk vs. PAH; [†]P<0.05, ^{†††}P<0.0001 for Controls vs. High Risk

CMR parameter	RHC parameter	Rest	Adenosine		
			70 mcg/kg/min	140 mcg/kg/min	210 mcg/kg/min
meanPAvel	CI	0.66***	0.59**	0.72****	0.69***
	mPAP	-0.76****	-0.80****	-0.77****	-0.77****
	PVR	-0.86****	-0.83****	-0.86****	-0.86****
Pulsatility	CI	0.65***	0.58**	0.44*	0.35
	mPAP	-0.69****	-0.71****	-0.74****	-0.62**
	PVR	-0.74****	-0.76****	-0.72****	-0.57**

Table 3: Correlation coefficients for phase-contrast CMR parameters vs. haemodynamic parameters at rest and during adenosine infusion (for PAH and High Risk groups).

*P<0.05, **P<0.005, ***P<0.001, ****P<0.0001

Parameter	Intra-observer variability		Inter-observer variability	
	Mean bias	95% limits of agreement	Mean bias	95% limits of agreement
Pulsatility	10	-30 - 45	14	-40 - 63
MeanPAvel	-0.01	-0.21 - 0.19	-2.38e-7	-0.27 - 0.27

Table 4: Intra-observer and inter-observer variability of phase-contrast CMR measurements.

CHAPTER 4

THE PREDICTIVE CAPABILITIES OF A NOVEL CARDIAC MAGNETIC RESONANCE IMAGING DERIVED MARKER OF CARDIOPULMONARY RESERVE ON ESTABLISHED PROGNOSTIC SURROGATE MARKERS IN PATIENTS WITH PULMONARY VASCULAR DISEASE: RESULTS OF LONGITUDINAL PILOT STUDY

KEY WORDS:

- Pulmonary Circulation, Hyperaemia
- Prognosis
- Adenosine
- Blood Flow Velocity
- Hypertension, Pulmonary
- Pulmonary Artery
- Ventricular Dysfunction, Right
- Magnetic Resonance Imaging

STATEMENT OF AUTHORSHIP

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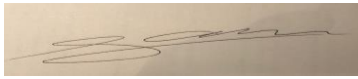
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- ii. permission is granted for the candidate to include the publication in the thesis; and
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ABSTRACT

Background

No unified method exists to effectively predict and monitor progression of PAH. We assessed the longitudinal relationship between a novel marker of cardiopulmonary reserve and established prognostic surrogate markers in patients with pulmonary vascular disease.

Methods and Results

Twenty participants with confirmed (n=14) or at high risk (n=6) for PAH underwent cardiac magnetic resonance imaging (CMR) at baseline and after ~6 months of guideline-appropriate management. Ten PAH participants underwent RHC within 48 hours of each CMR. RHC (mean pulmonary arterial pressure, mPAP; pulmonary vascular resistance index, PVRI; cardiac index, CI) and phase-contrast CMR (mean pulmonary arterial blood flow velocity, meanPAvel) measurements were taken at rest and during continuous adenosine infusion (70/140/210 mcg/kg/min). Initial meanPAvel's (rest and hyperemic) were correlated with validated surrogate prognostic parameters (CMR: RV ejection fraction, RVEF; RV end systolic volume indexed, RVESVI; RHC: PVRI, CI; biomarker: NT-pro brain natriuretic peptide, NTpBNP; clinical: 6-minute walk distance, 6MWD), a measure of pulmonary arterial stiffness (elastic modulus) and volumetric estimation of RV ventriculoarterial (VA) coupling. Changes in meanPAvel's were correlated with changes in comparator parameters over time.

At initial assessment, meanPAvel at rest correlated significantly with PVRI (inversely), CI (positively) and elastic modulus (inversely) ($R^2 > 0.37$, $P < 0.05$ for all), whereas meanPAvel at peak hyperaemia correlated significantly with PVRI, RVEF, RVESVI, 6MWD, elastic

modulus and VA coupling ($R^2 > 0.30$, $P < 0.05$ for all). Neither resting or hyperaemia-derived meanPAvel correlated with NTpBNP levels. Initial meanPAvel at rest correlated significantly with RVEF, RVESVI, CI and VA coupling at follow up assessment ($R^2 > 0.2$, $P < 0.05$ for all) and initial meanPAvel at peak hyperaemia correlated with RVEF, RVESVI, PVRI and VA coupling ($R^2 > 0.37$, $P < 0.05$ for all). Change in meanPAvel at rest over time did not show statistically significant correlation with change in prognostic parameters, while change in meanPAvel at peak hyperaemia did show a significant relationship with Δ RVEF, Δ RVESVI, Δ NTpBNP and Δ CI ($R^2 > 0.24$, $P < 0.05$ for all).

Conclusion

MeanPAvel during peak hyperaemia correlated with invasive, non-invasive and clinical prognostic parameters at different time points. Further studies with predefined clinical endpoints are required to evaluate if this novel tool is a marker of disease progression in patients with pulmonary vascular disease.

BACKGROUND

Pulmonary arterial hypertension (PAH) is characterised by a pathological increase in the resistance of the pulmonary circulation secondary to arterial wall remodelling, vasoconstriction and in situ thrombosis (pulmonary vascular disease, PVD). Progressive obliteration of the pulmonary vascular bed eventually leads to right ventricular (RV) failure and premature death. Therapeutic advances have contributed to better long-term outcomes but disease progression remains difficult to predict and objectively monitor necessitating new methods.

We previously demonstrated proof-of-concept for a novel non-invasive method to assess 'cardiopulmonary reserve' as it pertains to PAH by measuring the average pulmonary arterial blood flow velocity (meanPAvel) at rest and during standardised intravenous (IV) adenosine infusion using phase-contrast cardiac magnetic resonance imaging (CMR) [209]. This approach to 'stressing' the cardiopulmonary unit, whilst novel, was feasible, safe and simple, with the results confirming that meanPAvel at peak hyperaemia was an excellent functional correlate for cardiopulmonary reserve across a range of clinical risk phenotypes.

As a marker of cardiopulmonary reserve and with the advantage of ameliorating the impact of unrelated systemic processes on variables measured at rest, we hypothesised that meanPAvel at peak hyperaemia may provide prognostic information at initial assessment and during follow up. To evaluate this, we investigated the association of meanPAvel at rest and during peak hyperaemia with accepted RHC-derived, CMR-derived, biomarker and clinical prognostic surrogate markers in a cohort of patients with confirmed PAH or at high risk for incident PAH. Specifically, we hypothesised that relative to meanPAvel at rest, meanPAvel

at peak hyperaemia at initial assessment would be more closely associated with comparator parameters at initial and follow-up assessments, and that changes in meanPAvel at peak hyperaemia would correlate more closely with changes in these prognostic markers over time.

METHODS

We prospectively recruited participants with known or suspected PAH and a clinical indication for a right heart catheter (RHC) through a single tertiary-referral centre over a 14-month period (2013-2014). Patients were ineligible if they were <18 years old; pregnant; had known or suspected PAH due to congenital heart disease with left-to-right shunt, or portopulmonary hypertension; or, had a contraindication to CMR or IV adenosine. Right heart catheterization for suspected PAH was deemed clinically indicated by pulmonary hypertension experts following a comprehensive review of clinical status and ancillary investigations, in accordance with guidelines [40]. All participants provided written informed consent and protocols were approved by the Local Institutional Research Ethics Committee.

At initial assessment, all participants underwent a RHC and CMR with IV adenosine within 48hours, with PAH confirmed by haemodynamic criteria (mean pulmonary arterial pressure, mPAP, >25mmHg; mean pulmonary arterial wedge pressure, mPAWP, <15mmHg; and pulmonary vascular resistance, PVR >3 Woods units, WU). Participants with mPAWP > 15mmHg were excluded and did not undergo vasoreactive testing. Patients with PAH were invited to undergo repeat RHC and CMR with vasoreactivity testing and participants with suspected but excluded PAH (high-risk for incident PAH) invited to undergo repeat CMR with vasoreactive testing approximately 6 months after their initial study. Between

assessments, patients were administered guideline-appropriate management, where appropriate.

Right heart catheterization was performed via the right femoral vein with a 7 F Swan-Ganz thermodilution pulmonary artery catheter (Edwards Lifesciences, California, USA).

Systemic arterial pressures were monitored by arm cuff. Complete resting haemodynamic assessment was performed in accordance with current guidelines. Intravenous (IV) adenosine (Adenoscan, 3mg/mL, Astellas Pharma, Tokyo, Japan) was infused via peripheral vein at three increasing doses: 70-, 140-, and 210mcg/kg/min, and repeat haemodynamic assessment (mPAP/mPAWP/CI/PVR) conducted after a minimum of two minutes' infusion at each dose.

Cardiac magnetic resonance imaging was performed with a 1.5 Tesla magnet (Magnetom, Siemens, Erlangen, Germany) with images obtained at end-expiration. Volumetric and functional analyses were performed using steady-state free precession (SSFP) sequences of the atria and ventricles. Flow imaging through the main pulmonary artery was performed 1.5-2cm above the pulmonary valve using a velocity-encoded gradient echo sequence with an upper velocity limit of 150cm/sec, temporal resolution of 39ms and spatial resolution of 1.8x1.8x6mm. Adenosine was infused using the same protocol as during RHC, with flow imaging through the pulmonary trunk repeated after a minimum of 2 minutes at each adenosine dose. Analyses were performed offline using specialized software (CMR42, Circle Cardiovascular Imaging Inc., Calgary, Canada). Ventricular endocardial and epicardial contours were manually outlined at end-diastole and end-systole to permit calculation of ventricular volumes and myocardial mass by Simpson's method. Blood flow through the pulmonary trunk was measured by outlining the endovascular border at all 20 reconstructed cardiac phases permitting calculation of mean pulmonary arterial blood flow velocity

(meanPAvel), elastic modulus (pulse pressure x minA/(maxA-minA)) and a volumetric estimation of ventriculoarterial (VA) coupling (RVESV/RV stroke volume) [210]. All participants underwent 6-minute walk tests and had blood samples taken for NT-proBNP quantification, which was centrifuged within 1 hour for 10 minutes at 2800rpm, and stored at -80°C until analysis using an ELISA kit (USCN Life Science, Texas, USA).

Treatment Strategy

The objective was to assess the relative strength of association of meanPAvel measured at rest and at peak hyperaemia with comparator surrogate prognostic parameters. Treatment of participants with PAH was at the discretion of treating physicians and in line with current guidelines [7].

Comparator Surrogate Prognostic Parameters

Comparator surrogate prognostic parameters for initial and serial analyses were defined *a priori* as: 1) **CMR-derived**: right ventricular ejection fraction (RVEF) and right ventricular end systolic volume index (RVESVI); 2) **Biomarker**: NT-proBNP levels; 3) **RHC-derived**: pulmonary vascular resistance index (PVRI) and cardiac index (CI); 4) **Clinical**: six-minute walk distance (6MWD). In addition to these validated serial prognostic parameters, two other physiologically-relevant parameters were assessed: a marker of pulmonary arterial stiffness (elastic modulus; at initial assessment only), and volumetric estimation of VA coupling (at initial and follow-up assessments).

Statistical Analysis

Normally distributed continuous variables were expressed as means \pm standard deviations. Normality was assessed using the Shapiro-Wilk normality test. Scatterplots of univariate associations were reviewed and, where appropriate, Pearson correlation coefficients were used to explore the strength of linear relationships between meanPAvel at rest and during hyperaemia with comparator prognostic parameters and Spearman correlation coefficients were used to explore the strength of relationships that were curvilinear and where there was a significant departure from a Gaussian distribution. More specifically, meanPAvel measured at rest and during hyperaemia at the initial assessment were correlated with initial and follow up comparator parameters; and changes in meanPAvel at rest and during hyperaemia were correlated with changes in comparator parameters between initial assessment and follow up. Statistical analyses were performed using a software package (GraphPad Prism version 6.0, San Diego, California, USA), with a two-sided P value of <0.05 considered statistically significant.

RESULTS

Thirty-one participants were recruited (PAH n=19; suspected but excluded PAH: 'High Risk' n=12) of which 5 were ineligible (mPAWP>15mmHg n=4; claustrophobia n=1). 20 underwent repeat investigations (PAH n=14; High Risk n=6) after a median period of 8 months and 21 days (loss to follow-up in PAH group: death from RV failure n=1; geographical relocation n=1; declined n=1, and High Risk group: geographical relocation n=2; intercurrent illness n=1) and were included in the present study. Ten of the 14 PAH participants had both repeat CMR and RHC and 4 had CMR only (declined repeat RHC n=4). Demographic and clinical characteristics of participants are presented in Table 1, with suspected but excluded PAH participants considered high risk for incident PAH on the basis

of the high prevalence of connective tissue disease, unaccounted for exertional breathlessness with disproportionately reduced lung diffusing capacity, elevated NTpBNP levels in the absence of left ventricular myocardial disease or renal dysfunction, elevated estimated pulmonary arterial systolic pressure by transthoracic echocardiography >40mmHg (n=6) and 'borderline' abnormal resting haemodynamics (n=2 with rest mPAP of 21-24mmHg) [42].

Mean absolute values of comparator, investigational and other relevant parameters, and their relative changes over time, are summarised in Table 2.

Response to adenosine

The haemodynamic and meanPAvel changes in response to adenosine are shown in Figure 1. Systemic blood pressure (SBP) did not change significantly during adenosine (systolic SBP - $6\pm 13\%$ and $0\pm 18\%$ for PAH and High Risk groups respectively, $P=0.38$ and 0.23 respectively – not displayed graphically). There was minimal intra-observer and interobserver variability for meanPAvel measures (mean bias: -0.01 and -0.0000238 ; 95% limits of agreement: $-0.21-0.19$ and $-0.27-0.27$ respectively) at all doses.

Correlation between mean pulmonary arterial blood flow velocities and prognostic parameters measured at initial assessment

Correlation coefficients are presented in Table 3. MeanPAvel at rest showed strong positive correlation with CI and very strong negative correlation (curvilinear) with PVRI. There was moderate but statistically insignificant correlation with CMR indices (negative with RVESVI and positive with RVEF, Figure 3) and 6MWD (positive), but no correlation with NTpBNP. Elastic modulus, a measure of the pressure change required to drive a relative change in the PA lumen size, showed strong negative correlation with meanPAvel at rest. Volumetric

estimation of ventriculoarterial coupling showed moderate strength negative correlation with meanPAvel at rest. Therefore a high effective arterial elastance: maximal systolic elastance ratio (estimated here by CMR-derived RVESV/RVSV), suggestive of RV-pulmonary uncoupling, was associated with lower meanPAvels. Relative to meanPAvel at rest, meanPAvel at hyperaemia correlated more closely with all parameters except RHC-derived CI at rest and NTpBNP.

Given the weaker association between CI and meanPAvel at peak hyperaemia, compared to meanPAvel at rest, we assessed the relationship between meanPAvel's and haemodynamic measurements made during hyperaemia. In this regard, meanPAvel at rest and during hyperaemia correlated closely with the maximum CI ($R=0.60$, $P=0.005$ and $R=0.65$, $P=0.002$ respectively) and minimum PVRI ($R=-0.67$, $P=0.001$ and $R=-0.74$, $P=0.0002$ respectively) during adenosine challenge. This is consistent with earlier work demonstrating close correlation between meanPAvel and invasively-derived haemodynamics at rest and during adenosine infusion [211].

Correlation between initial mean pulmonary arterial blood flow velocities and follow up prognostic parameters

MeanPAvel measured at rest showed moderate strength statistically significant correlation with RVEF (Figure 3), RVESVI and VA coupling at follow up. Strong negative correlation with follow up PVRI and positive correlation with follow up CI were found. In contrast, there was weak and statistically insignificant correlation with follow up NTpBNP levels and no correlation with 6MWD. MeanPAvel measured during peak hyperaemia correlated more closely with all parameters except resting CI ($R = 0.67$ vs. 0.59 for meanPAvel at rest vs. peak hyperaemia), 6MWD and NTpBNP. Correlation coefficients are presented in Table 4.

Correlation between changes in mean pulmonary arterial blood flow velocities and changes in comparator prognostic parameters

There were no statistically significant associations between change in meanPAvel measured at rest and change in comparator prognostic parameters, although statistical power was reduced with regard to RHC-derived parameters owing to fewer follow-up invasive procedures (n=14 vs. n=10). In contrast, change in meanPAvel measured at hyperaemia correlated moderate-strongly with all comparator prognostic parameters except 6MWD, PVRI and VA coupling (R=0.30, P=0.30/R=-0.39, P=0.27/R=-0.44, P=0.06 respectively). Similar trends were seen when only confirmed PAH participants were analysed. Correlation coefficients are presented in Table 5.

Despite its routine use, 6MWD has many limitations as a serial prognostic marker (e.g. day-to-day variation, learning effect and comorbidities), with registry data showing a decline in 6MWD confers a worse prognosis but a rise in 6MWD has no impact on survival [212]. In those participants with a decline in 6MWD (N=9) in the present study, correlation with change in meanPAvel at hyperaemia improved but statistical significance was not met (R = -0.52, P = 0.22) possibly due to the low sample size.

DISCUSSION

Blood flow velocity through the main pulmonary artery is thought to decline in PAH due to impeded passage of cardiac output through remodelled microcirculation, dilation of proximal pulmonary vessels and eventually, impaired RV function [82]. Not surprisingly therefore, mean pulmonary arterial blood flow velocity (meanPAvel) measured at rest has been shown

to correlate with measures of RV function, arterial load and exercise capacity, which are all important determinants of prognosis in PAH [82] [78] [83] [81] [213]. This study adds to our knowledge by demonstrating correlation between our non-invasive measure of ‘cardiopulmonary’ reserve (meanPAvel at peak hyperaemia, [211]) and validated invasively and non-invasively derived surrogate prognostic markers at baseline and six month follow-up. This marker was superior to meanPAvel when measured at rest. These data would support further studies being undertaken with hard clinical endpoints, to establish if this novel marker would provide incremental prognostic information in patients with PAH or at high risk for incident PAH.

PAH is principally defined by the plexogenic pulmonary vasculopathy that underlies its genesis, increasing RV afterload and stress. These two compartments (the RV and pulmonary vasculature) are integrally related and not surprisingly therefore, measures of RV afterload (static e.g. PVR, and oscillatory e.g. pulmonary arterial stiffness, components), stress (e.g. NTpBNP), and functional adaptation (e.g. RVEF, RVESVI) all provide independent prognostic information. In isolation however, each parameter tends to provide a limited picture of the state of the ‘cardiopulmonary’ unit as a whole. In our study, meanPAvel at peak hyperaemia correlated significantly with measures of RV afterload (e.g. PVRI and elastic modulus), RV functional adaptation (e.g. RVEF/RVESVI) and VA coupling (defined as the ratio of maximal systolic RV elastance: effective arterial elastance), suggesting a capacity to simultaneously interrogate the pulmonary vasculature, the RV, and their interaction in a simple, non-invasive fashion. The capacity to assess the RV and pulmonary vasculature as a collective unit may translate to improved ability to discriminate changes in ventricular function, arterial load, or both when compared to, for example, RVEF, a serial

CMR measure that conveys consistent prognostic information in PAH patients [132, 133, 210].

'Traditional' prognostic parameters are generally measured at rest and are subject to the influence of unrelated systemic factors such as anxiety, hypertension and sedation which may be compounded by serial assessment. Moreover, they lack the capacity to assess physiological reserve, that is, they provide a static measure of a dynamic disease process. Our protocol, like other physiological stress tests, negates the influences of unrelated systemic factors whilst permitting assessment of reserve as it pertains to PAH, that is, the degree of functional pulmonary circulation recruitable by endothelial independent mechanisms with IV adenosine ('vascular reserve') and the capacity of the RV to increase blood flow in response to downstream circulatory recruitment ('RV reserve': chronotropic/inotropic). We postulate that the generally closer association found between meanPAvel at peak hyperaemia with validated prognostic parameters, compared with meanPAvel measured at rest, was due to the capacity to assess the cardiopulmonary unit as whole and its' reserve while ameliorating the impact of unrelated systemic processes.

Acknowledging the above, not all validated prognostic comparators correlated significantly with meanPAvel at peak hyperaemia. Most notably, NTpBNP levels measured at initial assessment did not correlate with meanPAvel measured at the same assessment or at follow-up, although the relative change in NTpBNP correlated with the change in meanPAvel at peak hyperaemia over time. Previous studies have demonstrated association between baseline NTpBNP levels, resting haemodynamics, lung function, peak VO₂ and prognosis, CMR-derived measures of RV function, and association between change in NTpBNP over time and survival [214-217] [218, 219]. The discordant findings of strong correlation between

meanPAvel at peak hyperaemia, resting haemodynamics and markers of RV functional adaptation (RVEF, RVESVI) but no correlation with NTpBNP levels in absolute terms were unexpected and difficult to explain biologically: they may therefore reflect a statistical anomaly. A lack of correlation between change in meanPAvel and change in PVRI over time was likely due to the small sample size undergoing repeat RHC (N=10) or, as earlier discussed, the impact of unrelated systemic factors on resting measures that may compound with serial assessment. No correlation between change in meanPAvel at peak hyperaemia and change in 6MWD over time may reflect the inherent limitations of serial 6MWDs and is in keeping with registry data showing a decline in 6MWD is prognostically relevant whereas an increase is not [220]. Closer association between these variables in the subset of patients that experienced a decline in 6MWD (n=9: R=0.59, P=0.22) is in keeping with our other findings, acknowledging that statistical significance was not met, likely due to a small sample size.

Study limitations

The major limitations are the small sample size, single-centre study design, and lack of clinical outcome data. Findings from this study should be considered hypothesis generating. Repeat vasoreactivity testing via invasive or non-invasive protocols is novel, with this study designed to explore its potential clinical utility by assessing relative strength of association across a range of validated prognostic parameters. Whether meanPAvel provides independent prognostic information cannot be determined. Evidence for potential clinical utility of repeat standardised vasoreactivity testing using this protocol is provided by the combination of physiologically appropriate correlation across a range of relevant CMR-derived, RHC-derived, biomarker and clinical variables; and correlation of generally greater

strength at hyperaemia compared to rest. Larger studies are required to confirm these findings.

CONCLUSION

Mean pulmonary arterial blood flow velocity measured using CMR during non-invasive standardised pulmonary vasoreactive testing correlated moderate-strongly with a range of CMR-derived, RHC-derived, biomarker and clinical prognostic variables at initial assessment and at follow up. Change in meanPAvel at peak hyperaemia on repeat vasoreactivity testing correlated with change in these variables over time, although correlation was generally weaker. Compared with meanPAvel measured at rest, correlation was generally stronger with meanPAvel measured at peak hyperaemia. The novel parameter of meanPAvel at peak hyperaemia may provide prognostic information by simultaneously interrogating the pulmonary vasculature and RV functional capacity whilst ameliorating the impact of unrelated systemic processes, although larger trials are required to confirm these findings.

FIGURES

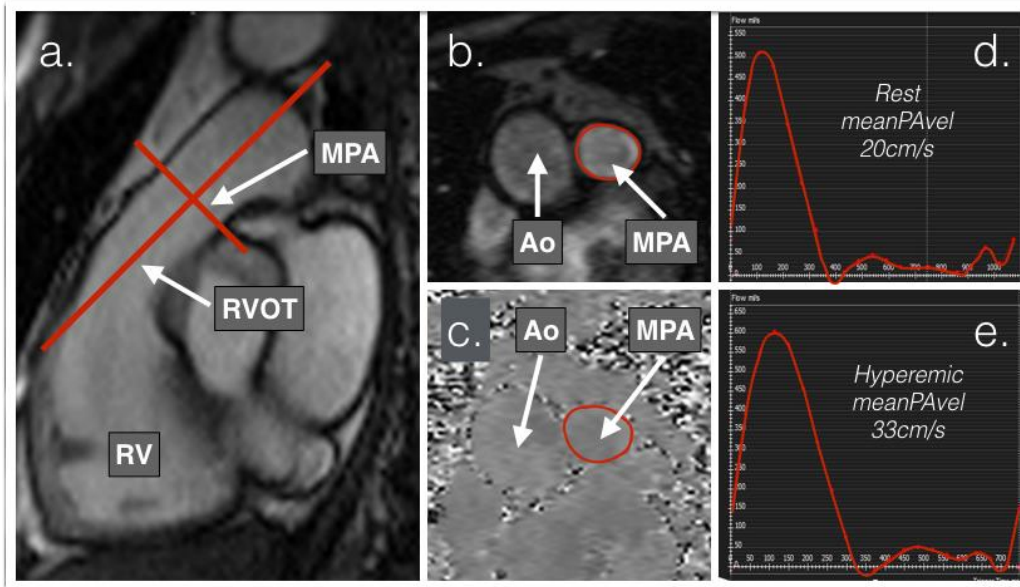


Figure 1: Reference sequences for main pulmonary arterial (MPA) phase contrast imaging were two double-oblique orthogonal views along the main axis of the pulmonary trunk (a.). The endocardial border of the MPA was manually outlined at all 20 reconstructed cardiac phases (b. and c.) permitting flow velocity profiles to be generated at rest and hyperaemia (d. and e. respectively: flow velocity in ml/s on y axis and time in milliseconds on x axis). MeanPAvel was calculated as the average blood flow velocity across all cardiac phases. Ao = aorta, RV = right ventricle, RVOT = right ventricular outflow tract.

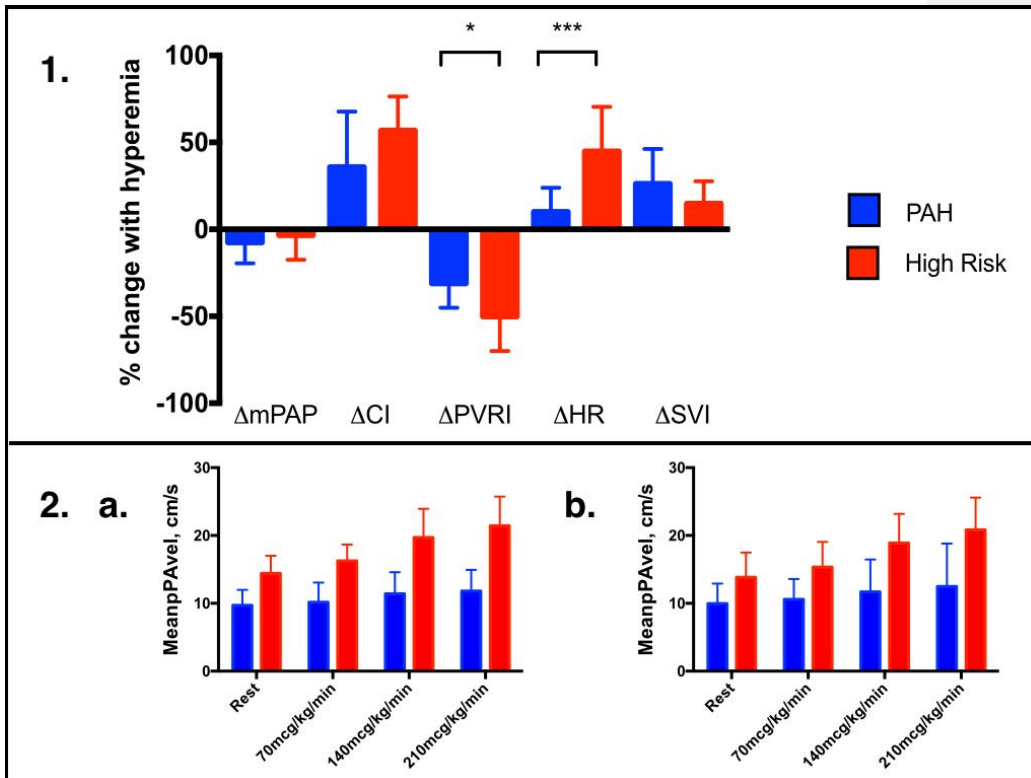


Figure 2: Changes in RHC-derived haemodynamics (panel 1) and CMR-derived meanPAvel (initial assessment, panel 2a; follow-up assessment, panel 2b) in response to adenosine in PAH and High Risk participants. At RHC, intravenous adenosine produced a dose dependent reduction in PVRI driven predominantly by a higher CI rather than a lower mPAP or transpulmonary gradient (not illustrated). At CMR, these changes were reflected by a dose-dependent rise in meanPAvel. Changes were more pronounced in High Risk participants relative to those with PAH.

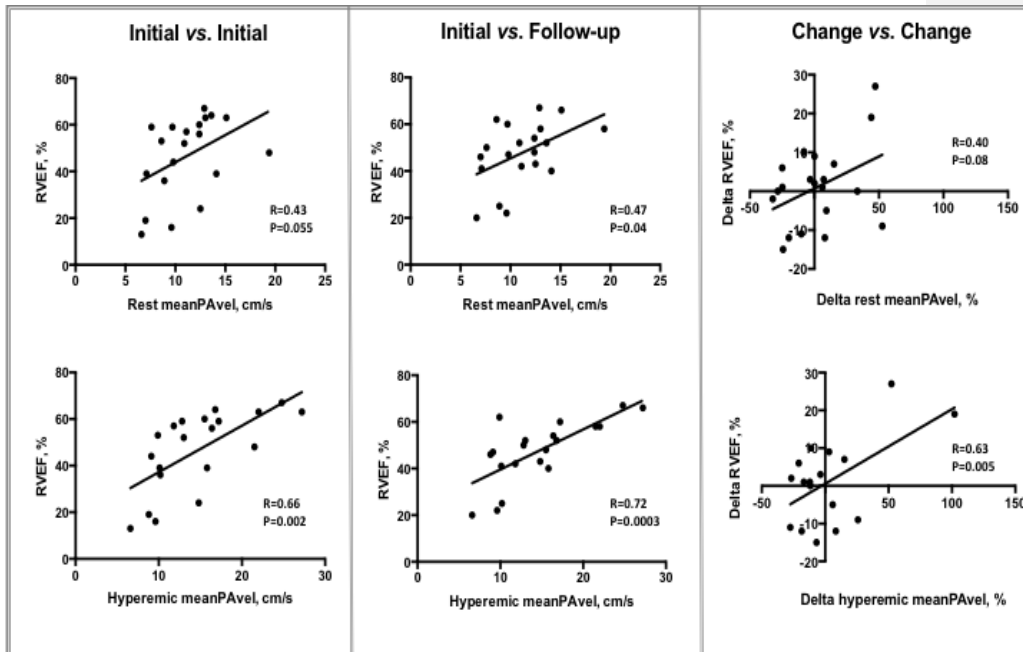


Figure 3: Scatterplots illustrating the relationship between RVEF, meanPAvel at rest and meanPAvel at hyperaemia at different time points.

Initial = at initial assessment; Follow-Up = at follow-up assessment; Change = change between initial and follow-up assessments; R = Pearson correlation coefficient.

TABLES

	PAH	High Risk	P value
Participants, n	14	6	
Female (%)	11 (79)	5 (83)	0.98
Age, years	54±14	63±7.6	0.18
BSA, m ²	1.79±0.25	1.83±0.17	0.79
PAH aetiology (%)			
Idiopathic	10 (71)		
CTD-associated	4 (29)		
Coexisting conditions (%)			
Diabetes mellitus	2 (14)	0 (0)	<0.05
Smoker	1 (12)	0 (0)	<0.05
CTD	5 (36)	5 (83)	<0.05
NYHA functional class (%)			
II	3 (21)	6 (100)	<0.05
III	11 (79)	0 (0)	<0.05
Therapy, initial/follow-up (%)			
Phosphodiesterase-5 inhibitor	1 (7)/0 (0)		
Combination	13(93)/14(100)		
Creatinine, µmol/L	84±21	83±34	0.96
D _L CO, %	55±15	58±19	0.76
%FVC/%D _L CO	1.41±0.41	1.38±0.27	0.92

Table 1: Demographic and clinical characteristics of participants.

	Parameter	Group	Initial	Follow-up	Change (%)
CMR-derived	RVEF (%)	PAH	41±17	43±13	18±47
		High risk	60±7	59±6	-1±13
	RVESVI (ml/m ²)	PAH	50±26	54±25	17±49
		High risk	24±7	25±8	4±19
	meanPAvel at rest (cm/s)	PAH	9.7±2.3	10.0±3.0	5±27
		High risk	14.4±2.6	13.9±3.6	-4±23
	meanPAvel during hyperaemia (cm/s)	PAH	12.2±3.3	12.9±6.3	6±32
		High risk	21.5±4.3	19.8±4.9	-8±8
	RVEDVI (ml/m ²)	PAH	84±19	91±22	8±15
		High risk	61±14	61±17	0±13
LVEF (%)	PAH	65±11	68±9	5±21	
	High risk	69±4	66±6	-3±9	
RHC-derived	CI (L/min/ m ²)	PAH	2.35±0.76	2.59±0.81	13±19
		High risk	2.80±0.55	n/a	n/a
	PVRI (WU/ m ²)	PAH	5.5±3.2	5.3±3.4	11±47
		High risk	1.0±0.4	n/a	n/a
	mPAP (mmHg)	PAH	46±16	48±14	9±24
		High risk	19±4	n/a	n/a
mPAWP (mmHg)	PAH	10±2	8±5	3±30	
	High risk	10±3	n/a	n/a	
Clinical/Biomarker	NTpBNP (pg/ml)	PAH	2310±1354	2740±1361	71±200
		High risk	1345±718	1289±693	-3±13
	6MWD (m)	PAH	421±81	435±62	7±18
		High risk	484±71	437±94	-3±8

Table 2: Absolute values and relative change in comparator (bold text) and investigational parameters (mean \pm SD).

Comparator parameter	meanPAvel at rest	P value	meanPAvel during hyperaemia	P value
RVEF	0.43 [-0.01 to 0.74]	0.055	0.66** [0.31 to 0.85]	0.002
RVESVI	-0.38 [-0.71 to 0.09]	0.10	-0.70*** [-0.88 to -0.36]	0.0006
NTpBNP	-0.19 [-0.61 to 0.30]	0.44	-0.21 [-0.62 to 0.29]	0.40
PVRI	-0.81**** [-0.93 to -0.57]	<0.0001	-0.88*** [-0.95 to -0.70]	<0.0001
CI	0.65** [0.30 to 0.85]	0.002	0.41 [-0.03 to 0.72]	0.07
6MWD	0.43 [-0.04 to 0.74]	0.07	0.58** [0.17 to 0.82]	0.009
Elastic modulus	-0.61** [-0.83 to -0.23]	0.004	-0.65** [-0.85 to -0.29]	0.001
VA coupling	-0.49* [-0.77 to -0.05]	0.03	-0.75*** [-0.90 to -0.46]	0.0001
Maximum CI	0.60** [0.22 to 0.83]	0.005	0.65** [0.29 to 0.85]	0.002
Minimum PVRI	-0.76** [-0.90 to -0.47]	0.001	-0.74*** [-0.94 to -0.62]	0.0002

Table 3: Correlation coefficients investigating the relationship between meanPAvel and comparator parameters measured during initial assessment.

[95% confidence interval]

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Comparator parameter	meanPAvel at rest	P value	meanPAvel during hyperaemia	P value
RVEF	0.47* [0.04 to 0.76]	0.04	0.72*** [0.41 to 0.88]	0.0003
RVESVI	-0.53* [-0.79 to -0.10]	0.02	-0.71*** [-0.88 to -0.37]	0.0005
NTpBNP	-0.40 [-0.73 to 0.06]	0.09	-0.42 [-0.73 to 0.04]	0.07
PVRI	-0.62* [-0.89 to -0.01]	0.05	-0.70* [-0.92 to -0.15]	0.02
CI	0.67* [0.11 to 0.90]	0.03	0.59 [-0.02 to 0.88]	0.06
6MWD	0.23 [-0.26 to 0.63]	0.34	0.10 [-0.39 to 0.54]	0.71
VA coupling	-0.46* [-0.75 to -0.01]	0.04	-0.71*** [-0.88 to -0.37]	0.0005

Table 4: Correlation coefficients investigating the relationship between meanPAvel measured during initial assessment with follow-up comparator parameters.

[95% confidence interval]

*P<0.05

**P<0.01

***P<0.001

Comparator parameter	meanPAvel at rest	P value	meanPAvel during hyperaemia	P value
Δ RVeF	0.40 [-0.05 to 0.72]	0.08	0.63** [0.23 to 0.85]	0.005
Δ RVeSVI	-0.23 [-0.61 to 0.24]	0.34	-0.50* [-0.78 to -0.05]	0.03
Δ NTpBNP	-0.30 [-0.71 to 0.20]	0.26	-0.52* [-0.82 to -0.01]	0.04
Δ PVRI	-0.07 [-0.64 to 0.55]	0.84	-0.39 [-0.82 to 0.32]	0.27
Δ CI	0.50 [-0.14 to 0.85]	0.12	0.62* [-0.02 to 0.90]	0.05
Δ 6MWD	0.26 [-0.25 to 0.66]	0.31	0.30 [-0.27 to 0.72]	0.30
Δ VA coupling	-0.28 [-0.64 to 0.18]	0.23	-0.44 [-0.74 to 0.02]	0.06

Table 5: Correlation coefficients investigating the relationship between changes in meanPAvel and changes in comparator parameters over time.

[95% confidence interval]

*P<0.05

**P<0.01

CHAPTER 5

THE RELATIONSHIP BETWEEN CORONARY FLOW RESERVE, MYOCARDIAL BLOOD SUPPLY:DEMAND BALANCE, DISEASE SEVERITY, AND CARDIOPULMONARY RESERVE IN PATIENTS WITH PULMONARY VASCULAR DISEASE

KEY WORDS:

- Hypertension, pulmonary
- Magnetic resonance imaging
- Haemodynamics
- Right ventricle
- Coronary
- Coronary sinus

STATEMENT OF AUTHORSHIP

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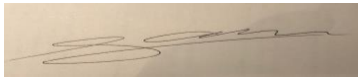
Name of Principal Author (Candidate)	Timothy James Gregory BAILLIE		
Contribution to the Paper	Protocol design, data collection and analysis, preparation of manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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ABSTRACT

Introduction

Right ventricular (RV) failure is an important cause of morbidity and mortality in pulmonary arterial hypertension (PAH). Pre-clinical studies implicate RV ischemia as a contributing cause but our understanding of the relationships between disease burden (resistive and pulsatile afterload, AL), coronary status, RV performance, and cardiopulmonary reserve in the clinical setting is limited.

Methods/Results

Patients with known or suspected PAH underwent right heart catheterization (RHC) and CMR with intravenous adenosine (210mcg/kg/min), and Healthy Controls (n=9) underwent CMR only. PAH was confirmed (n=17) or excluded (n=9, 'High Risk' group) by haemodynamic criteria. Coronary sinus flow reserve (CSFR) and RHC-derived indexes of myocardial blood supply:demand for the RV (PI/TTI) and LV (DPTI/SPTI) were measured. CSFR and PI/TTI had a significant direct relationship ($R=0.59$, $P<0.03$) and both were significantly lower in the PAH group (CSFR: $P<0.05$ vs. High Risk and Controls; PI/TTI: $P<0.01$ vs. high risk). DPTI/SPTI was similar between PAH and high risk groups and not related to CSFR ($R=-0.13$). In PAH and High Risk groups, CSFR and PI/TTI correlated significantly with RV AL (distensibility/pulsatility $R^2\geq 0.21$, $P\leq 0.03$; pulmonary vascular resistance/mean pulmonary pressure $R^2>0.37$, $P<0.03$), RV remodelling (indexed RV mass/end diastolic and systolic volumes/right atrial volume $R^2\geq 0.37$, $P\leq 0.03$), RV performance (RV ejection fraction/ventriculoarterial coupling, $R^2\geq 0.52$, $P\leq 0.03$), and a functional correlate for cardiopulmonary reserve (CMR-derived mean pulmonary arterial

blood flow velocity during hyperaemia, $R^2 \geq 0.4$, $P \leq 0.03$). DPTI/SPTI did not correlate with these parameters.

Conclusion

CSFR and the myocardial supply:demand index for the RV are decreased in PAH and are directly related to disease severity (RV AL), RV remodeling/performance, and a functional correlate for cardiopulmonary reserve.

INTRODUCTION

Progressive pulmonary vasculopathy is the hallmark of pulmonary arterial hypertension (PAH), increasing both resistive and pulsatile components of right ventricular (RV) afterload (AL). The capacity of the RV to adapt to this load is an important prognostic factor. Initially, high wall stress promotes adaptive changes which enable matching of ventricular performance to arterial load. Progression of disease ultimately leads to maladaptive remodelling, characterized by chamber dilation, eccentric hypertrophy, systolic and diastolic dysfunction, and right heart failure (RHF). Pre-clinical studies implicate right ventricular ischemia as a contributing cause and potential treatment target [140, 165, 168, 188], but our understanding of the relationships between RV workload, RV myocardial perfusion, and RV performance in the clinical setting is limited.

In PAH, RV pressurization impacts both myocardial blood supply (reducing the pressure index pressure index, PI: difference between aortic and RV pressures throughout the cardiac cycle) and 'demand' (increasing wall tension which can be approximated by the tension time index, TTI: area under the RV pressure curve in systole). Autoregulation of the coronary bed and enhanced myocardial oxygen extraction can compensate but once exhausted, flow becomes solely dependent on forward pressure and susceptible to small additional perturbations in the supply:demand balance (e.g. exercise, tachycardia, progression of PVD). If coronary flow is insufficient, energy-dependent cellular processes will be downregulated to restore metabolic balance [164], leading to unsatisfactory RV contractile performance. Therefore, a greater understanding of these relationships may be clinically useful.

No single study has investigated the relationship between coronary micro- and macrovascular status, RV AL, RV remodelling/performance, and cardiopulmonary reserve in the clinical setting. The purpose of this study was to investigate these relationships across a spectrum of PVD severity using noncontrast phase-contrast (PC) cine cardiac magnetic resonance imaging (CMR) measurement of coronary sinus flow reserve (CSFR) [175] [176, 177], haemodynamic measurement of myocardial blood supply:demand indexes, haemodynamic and CMR measures of RV AL and RV remodelling/performance, and a functional correlate for cardiopulmonary reserve (mean velocity of blood flow in the main pulmonary artery measured during adenosine-induced hyperaemia, hyperemic meanPAvel) [209, 221]. We hypothesized that hyperemic coronary flow reserve (CSFR) and the myocardial blood supply:demand index for the RV would decline in accordance with RV AL, RV remodelling and performance, and cardiopulmonary reserve.

METHODS

Patients with known or suspected PAH and a clinical indication for a right heart catheter (RHC) were prospectively enrolled from a single tertiary-referral centre over a 14-month period (2013-2014). Exclusion criteria were <18 years old; pregnancy; known or suspected PAH due to congenital heart disease with left-to-right shunt, or portopulmonary hypertension; a mean pulmonary arterial wedge pressure (mPAWP) >15mmHg; stenosis >50% in any non-branch coronary artery; or, a contraindication to CMR or intravenous (IV) adenosine. The clinical decision to perform a RHC was made by treating pulmonary hypertension physicians in accordance with guideline-directed practices after review of appropriate ancillary investigations [222]. Age and sex matched healthy volunteers were also enrolled. The study protocol conforms to the ethical guidelines of the 1975 Declaration

of Helsinki as reflected in a priori approval by the Local Institutional Research Ethics Committee, and participants provided written informed consent.

Participants with known or suspected PAH underwent RHC and CMR with IV adenosine within 48 hours. PAH was confirmed by haemodynamic criteria (mean pulmonary arterial pressure [mPAP] >25mmHg, mPAWP <15mmHg, pulmonary vascular resistance [PVR] >3 Wood units [WU]). Healthy controls underwent CMR with IV adenosine only.

Right heart catheterization was performed via the right femoral vein using a 7 F Swan-Ganz thermodilution catheter (Edwards Lifesciences, California, USA). Standard resting haemodynamic measurements were made and cardiac output (CO) measured by thermodilution in triplicate. Systemic arterial pressures were measured throughout the procedure by arm cuff. Diagnostic coronary angiography was performed via the right femoral or radial artery (if not conducted at our institution within the preceding 3 years).

CMR images were obtained at end expiration using a 1.5 T magnet (Magnetom, Siemens, Erlangen, Germany). Steady-state free procession (SSFP) sequences of the atria and ventricles were acquired for volumetric/functional analyses. Phase-contrast (PC) imaging of the coronary sinus (CS; upper velocity limit of 50 cm/sec, temporal resolution of 8.6 milliseconds, 20 phase per cardiac cycle, Figure 1) and main pulmonary artery (PA; 1.5cm above the pulmonic valve; upper velocity limit of 150cm/sec, temporal resolution of 39ms, spatial resolution of 1.8x1.8x6mm, 20 phase per cardiac cycle) was performed at rest and during adenosine-stress (at a dose of 210mcg/kg/min). Analyses were performed offline using specialized software (CMR42, Circle Cardiovascular Imaging Inc., Calgary, Canada). Ventricular endocardial and epicardial borders were outlined manually at end-diastole and end-systole to calculate volumes and myocardial mass using Simpson's method. Atrial

volumes were measured at end of ventricular systole. The endovascular border of the CS and PA were manually outlined in all twenty reconstructed cardiac phases to calculate CS and PA flows. CSF was adjusted to biventricular mass to provide coronary blood flow per milligram of myocardium per minute. CSFR was calculated by dividing CSF measured during adenosine-induced hyperaemia (CSF_{stress} , ml/mg/min) by CSF at rest (CSF_{rest} , ml/mg/min). Mean pulmonary arterial blood flow velocity (meanPAvel) at hyperaemia was calculated by averaging velocities across all twenty reconstructed cardiac phases during adenosine-stress (hyperemic meanPAvel, cm/sec). Pulsatility and distensibility of the main PA were calculated at rest using the following formulae:

$$(1) \text{ Pulsatility} = [\text{maxPAarea} - \text{minPAarea}] / \text{maxPAarea} * 100$$

$$(2) \text{ Distensibility} = [(\text{maxPAarea} - \text{minPAarea}) / \text{PP} * \text{minPAarea}] * 100$$

where maxPAarea and minPA area are the maximum and minimum cross sectional areas of the PA during the cardiac cycle and PP is the pulmonary pulse pressure.

Myocardial blood supply:demand indexes were calculated using the following rationale and formulae:

1. Right ventricle (PI/TTI)

Coronary blood flow to the RV ('supply'; PI) occurs during systole and diastole and is the product of the coronary perfusion pressure (CPP) and duration of each phase. Systolic CPP (sCPP) reflects the difference between systemic systolic pressure (SSP) and RV systolic pressure (RVSP), and diastolic CPP (dCCP) the difference between systemic diastolic pressure (SDP) and RV diastolic pressure (RVEDP). Systolic time is typically fixed at ~200ms with the remaining time occupied by diastole:

- $PI = (sCPP \times \text{systolic time}) + (dCPP \times \text{diastolic time})$
 - $sCPP = (SSP - RVSP) \times 0.2s$
 - $dCPP = (SDP - RVEDP) \times ([60s/\text{heart rate}] - 0.2s)$

‘Demand’ is reflected by the area under the RV systolic pressure curve (TTI), the product of RV systolic pressure and systolic time:

- $TTI = RVSP \times 0.2s$

2. Left ventricle (DPTI/SPTI)

Coronary bloody flow to the LV (‘supply’; DPTI) occurs during diastole only and is the product of the CPP and diastolic time:

- $DPTI = (DSP - LVEDP) \times ([60s/\text{heart rate}] - 0.2s)$

‘Demand’ is the area under the LV systolic pressure curve (SPTI):

- $SPTI = SSP \times 0.2s$

Continuous variables are expressed as mean \pm SD. Normality was assessed using the Shapiro-Wilk normality test and outliers identified using the ROUT method (Q=1%). Unpaired t-tests were used to compare differences between groups with a two-sided *P* value of <0.05 considered statistically significant. RHC and CMR-derived parameters of interest were selected *a priori*: **RV AL** (resistive: mPAP, PVR; pulsatile: PA pulsatility and distensibility); **RV remodeling/performance** (all volume and mass measures indexed to BSA: RV mass [RVmassI], RA volume [RAvolI], RV end diastolic volume [RVEDVI], RV end systolic volume [RVESVI], RV ejection fraction [RVEF], and a volumetric estimation of ventriculo-arterial [VA] coupling [ratio of effective arterial elastance, *E_a*, to maximal systolic

ventricular elastance, E_{max} , calculated by: $RVESV/RV$ stroke volume] [210]; **cardiopulmonary reserve** (mean PA_{vel} during hyperaemia); **myocardial blood supply:demand indexes** (RV: PI/TTI , LV: $DPTI/SPTI$). Scatterplots were visualized and Pearson correlation coefficients used to explore strength of linear relationships between CSFR and normally distributed comparator parameters, and Spearman correlation coefficients used to explore strength of curvilinear relationships where there was a departure from Gaussian distribution. Relationships between CSFR and all comparator parameters were explored, whereas myocardial blood supply:demand indexes were related only to CMR-derived parameters and PVR (due to being directly intertwined with other RHC-derived parameters). To account for multiple comparisons, a P value <0.03 was considered statistically significant. Statistical analyses were performed using a software package (GraphPad Prism version 7.0, San Diego, California, USA).

RESULTS

Study Population

Forty-one participants (31 known or suspected PAH, 10 healthy controls) were recruited for this study. Five patients and 1 healthy control were excluded ($mPAWP >15\text{mmHg}$ $n = 4$; claustrophobia $n = 1$; intolerant to maximum dose adenosine $n = 1$). PAH was confirmed in 17 participants (PAH group) and excluded in 9 participants. For the purpose of this study, these participants were labelled 'high risk' for incident PAH (possible early PVD, 'High Risk' group) because of unexplained breathlessness ($n=9$), high prevalence of connective tissue disease (scleroderma $n = 5$; systemic lupus erythematosus $n = 1$), disproportionately reduced pulmonary diffusing capacity for carbon monoxide, elevated NT-pro brain natriuretic peptide levels without myocardial or renal dysfunction, and 'borderline' abnormal resting

haemodynamics (resting mPAP 21-24mmHg, n = 2)[42]. Demographic and clinical characteristics are presented in Table 1.

CSF and myocardial supply:demand indexes

CSFR was significantly lower in the PAH group compared with High Risk and Healthy Control groups (1.7 ± 1.2 vs. 2.9 ± 1.4 and 3.1 ± 1.1 , $P = 0.02$ and 0.006 respectively, Figure 1). There was no significant difference in CSFR between High Risk and Control groups. Mean CSF_{rest} was highest in the PAH group (1.0 ± 0.5 vs. 0.7 ± 0.3 and 0.8 ± 0.3 respectively, $P = NS$), although statistical significance was not met.

PI/TTI was significantly lower in the PAH group compared with the High Risk group (3.6 ± 2.1 vs. 10.8 ± 2.3 , $P < 0.0001$) but there was no difference in DPTI/SPTI (1.6 ± 0.4 vs. 1.6 ± 0.4 , $P = NS$, Table 2 and Figure 3). Considering 'supply' (PI and DPTI) and 'demand' (TTI and SPTI) independently, there were significant differences in both PI and TTI between groups ($P < 0.002$ for both, data not presented) but no differences in DPTI or SPTI ($P = NS$ for both, data not presented).

A significant positive linear correlation was evident between CSFR and PI/TTI ($R = 0.59$, $P = 0.002$), but no correlation between CSFR and DPTI/SPTI ($R = -0.13$, $P = 0.54$).

Comparator parameters

Absolute values and correlation coefficients are presented in Table 2. All parameters were significantly different between PAH and High Risk groups except RVEDVI ($P = 0.25$) and DPTI/SPTI ($P = 0.79$). CSFR correlated significantly with all parameters except DPTI/SPTI. Relationships with mPAP, PVR, RVmassI, RAvolI, RVESVI, RVEF, and VAcoupling were

non-linear, with perturbations in comparator parameters becoming much more marked below a CSFR of ~ 2 , whereas RVEDVI and hyperemic meanPAvel varied in a linear fashion with CSFR (Figure 4).

PI/TTI correlated significantly with all comparator parameters except RVEDVI ($P=0.04$) (Table 2). Like CSFR, PI/TTI varied in a non-linear fashion with PVR, RVmassI, RAvolI, RVESVI, RVEF, and VAcoupling, with marked perturbations found in subjects with a PI/TTI of $< \sim 5$ (Figure 5). Pulsatility and hyperemic meanPAvel declined linearly with PI/TTI. No correlation between DPTI/SPTI and comparator parameters was found ($R < 0.30$, $P > 0.1$ for all, data not presented).

DISCUSSION

Prognosis in PAH is closely linked to the capacity of the RV to maintain cardiac output in the presence of a rising afterload. Clinical symptoms such as a declining functional capacity, syncope and chest pain in patients with PAH can signal that cardiac reserve is exhausted. Pre-clinical and clinical studies suggest that inadequate coronary blood flow to a severely pressurized RV may contribute to contractile dysfunction and RV failure [140, 165, 168-171, 178, 188]. The present study supports this by showing that coronary flow reserve and the right ventricular myocardial blood supply:demand index are lower in PAH subjects, and are related to resting measures of afterload, remodelling, and performance of the RV. It adds to prior knowledge by demonstrating a non-linear, threshold-type relationship between coronary perfusion and ventriculo-arterial coupling (ratio of effective arterial elastance to maximal systolic ventricular elastance), and a linear relationship with a functional correlate for cardiopulmonary reserve. These findings suggest that inadequate RV coronary perfusion

may contribute to a limited cardiopulmonary reserve at an earlier disease stage, and to uncoupling of the RV-PA circuit when PVD is more severe.

Right ventricular workload rises with PVD progression, increasing metabolic and (therefore) oxygen demands. Coronary perfusion must increase to meet these demands and avoid pump failure. However, unique physiology dictates that RCA driving pressure is concomitantly reduced by RV pressurization (lower systolic and diastolic pressure gradients between the aorta and RV which may be compounded by systemic hypotension in advanced disease), and systolic epicardial RCA flow may be restricted by compression [169], further limiting supply. Clinical and experimental studies suggest that compensatory mechanisms such as microvascular dilation [140, 188, 223], increased myocardial capillarization [153], or improved mechanical efficiency [180], have limited capacity to overcome coronary supply:demand imbalance so that, inevitably, contractile function must be sacrificed to maintain perfusion-contraction matching [164, 224]. This sequence of events has been confirmed in experimental studies (with the capacity to systematically manipulate physiologic parameters) but limited evidence of this is available in the clinical setting.

By examining relationships between RV AL, RV performance/ and remodelling, and cardiopulmonary reserve across a range of PVD severities we could explore their interaction in relation to disease progression. In earlier disease (that is, lower resistive afterload or PVR), PI/TTI and CSFR decreased in relation to the pulsatile but not resistive RV AL. This is consistent with the well-defined inverse relationship between resistive (resistance, R) and pulsatile (compliance, C) afterloads of the pulmonary circulation (which dictates that C is compromised to a greater degree than R in early PVD), and the fact that pulsatile afterload contributes significantly to total RV AL (~25% of total AL in contrast to ~10% in the

systemic circulation) [56, 66]. With disease progression, we observed a continued decline in CSFR and PI/TTI as well as evidence of RV remodelling (increased RVEDVI, RVESVI, and RVmassI), but relatively preserved RV performance (RVEF, VAcoupling). We postulate that compensatory mechanisms (e.g. coronary vasodilation, increased capillarization, RV hypertrophy mitigating wall tension etc.) at this point are sufficient to meet the higher metabolic demands of the pressurized RV, enabling contractility to overcome AL at rest. The higher myocardial blood flow at rest in the PAH group, while not statistically significant, supports this and is consistent with earlier work by Dubiel *et al.* which demonstrated higher myocardial blood flow and lower coronary vascular resistance in the RV of patients with moderate PH [185].

In more advanced disease, we observed a consistent inflection point (at a PI/TTI <5 and CSFR \sim 2) after which disease progression had little further impact on PI/TTI or CSFR, but a marked impact on RV remodelling and performance. This is best illustrated by examining the relationship between coronary perfusion (both CSFR and PI/TTI) and ventriculo-arterial coupling (Figure 3), which has the advantage of reflecting changes in total RV AL, RV performance, or both [210, 225]. We hypothesize that at (and beyond) this inflection point or 'threshold', compensatory mechanisms in the coronary vascular bed are maximized so that small additional perturbations in perfusion (e.g. progression of PVD) render oxygen supply inadequate for the metabolic needs of the RV, leading to contractile dysfunction and uncoupling of E_{max} from E_a , but maintenance of perfusion-contraction matching. Pre-clinical studies have shown that supraphysiologic coronary perfusion can restore RV performance despite a severely elevated AL [140, 165, 188], but clinical studies exploring direct manipulation of coronary perfusion parameters in ambulatory PAH patients are lacking.

Mean PA blood flow velocity at hyperaemia performs as a functional correlate for cardiopulmonary reserve, incorporating both the vascular ('vascular' reserve) and ventricular ('RV reserve', inotropic and chronotropic) components of the right heart-lung unit [209, 221]. Unlike the curvilinear relationships between coronary perfusion, RV AL, and RV performance/remodelling parameters measured at rest, we found a linear inverse relationship between mean PA blood velocity at hyperaemia and coronary perfusion parameters (Figure 3). We postulate that myocardial oxygen delivery may not increase sufficiently to meet the higher metabolic requirements of physiologic stress, leading to relative contractile dysfunction and a limited 'RV reserve', even in subjects without severe PVD as determined by traditional resting RHC- and CMR-derived parameters. Gomez *et al.* found scintigraphic evidence of exercise-induced subendocardial RV ischemia in a subgroup of PAH patients with severe disease [170], but to the best of our knowledge, the present study is the first to link RV coronary perfusion perturbations with physiologic reserve at an earlier disease stage.

Implications

Findings from the present study suggest that in advanced PVD, RV contractile dysfunction and uncoupling of the RV-PA circuit may in part be due to an insufficient myocardial blood supply (and hence oxygen supply) relative to demand. Given PVD is progressive despite pulmonary vasodilator therapy, and lung transplantation is not uniformly suitable or available, therapeutically targeting determinants of RV myocardial oxygen supply, demand, and utilization by means supplementary to lowering PVR may improve RV metabolic and contractile potential, even in the face of a persistently high AL. Since heart rate is a major determinant of RV oxygen demand [179], selective sinus node I_f channel inhibition (ivabradine) may be beneficial and is supported by small studies in PAH and PH-lung disease patients, although mechanisms underlying the observed clinical improvement are not clear

[226, 227]. Increasing myocardial capillary density [228], ameliorating autonomic dysregulation [229], and mitigating mitochondrial dysfunction [230, 231] may be alternative approaches. Lastly, easily accessible measures of RV perfusion parameters (e.g. PI/TTI) may assist in the assessment of clinical risk in PAH patients.

Study Limitations

The small sample size, single-centre design, and lack of clinical outcome measures are major limitations. It is not possible to determine causation (between RV AL and coronary perfusion, and coronary perfusion and RV remodelling/performance/cardiopulmonary reserve) from our data, as was illustrated in pre-clinical studies. Our findings are consistent with existing pre-clinical and clinical work and are supported by sound pathophysiologic rationale, but should be considered hypothesis generating until larger trials powered to explore the independent clinical value of measuring/manipulating coronary perfusion are conducted. In calculating PI/TTI, we did not consider the wall tension or oxygen carrying capacity of the blood (haemoglobin level), systemic BP was measured by arm cuff rather than invasively, and a fixed systolic time of 200ms was used in place of a calculated systolic time interval using regression equations proposed by Weissler *et al* [232]. While systematic error may have been introduced, observed between-group differences or correlation coefficients should not have impacted. Furthermore, Wong *et al.* showed that adding wall stress to HR and PASP (which are incorporated in our PI/TTI calculation) did not improve association with RV myocardial oxygen consumption [179], and haemoglobin levels were similar between patient groups. Minor epicardial coronary artery disease or microvascular dysfunction unrelated to PAH/PVD (e.g. diabetes mellitus, connective tissue disease) may have influenced CSFR measures. This impact is thought to be small as subjects with epicardial stenoses >50% were excluded, and no difference in CSFR between CTD-PAH and iPAH was found (data not

presented). Finally, the coronary sinus does not transport coronary efflux from all vascular territories, including the anterior cardiac veins. Our results support CSFR relating predominantly to alterations in RV myocardial blood supply/demand in this population given the lack of association between LV-derived parameters across both groups.

CONCLUSION

The present study provides insights on the relationships between RV AL, micro- and macrovascular coronary status, RV remodelling/performance, and cardiopulmonary reserve, demonstrating that RV perfusion declines early in relation to a rising pulsatile afterload, a declining cardiopulmonary reserve, but relatively preserved RV structure and performance, whereas in advanced PAH, minor perturbations in coronary perfusion are associated with a dramatic decline in RV performance and uncoupling of the RV-PA system. Directly targeting RV perfusion parameters may therefore be of therapeutic benefit.

FIGURES

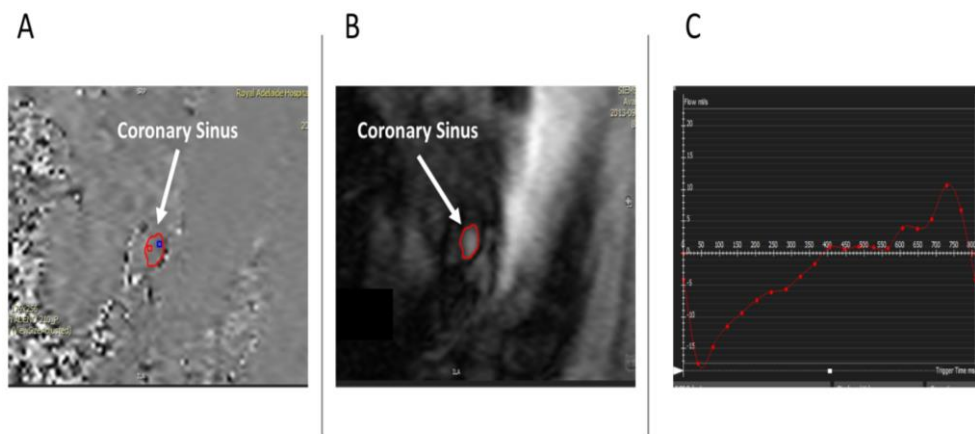


Figure 1: Flow imaging of the coronary sinus (CS). The CS was identified in the basal short axis SSFP images within the atrioventricular groove. The reference plane for flow imaging was parallel to the long axis of the heart, about 0.5cm from the CS ostium. Velocity encoded images were acquired (A and B) at rest and with adenosine (210mcg/kg/min). Coronary sinus flow was measured by tracing the endovascular border at all 20 reconstructed cardiac phases, generating a flow velocity profile (C, where the x axis is time in milliseconds and y axis flow velocity in meters/second).

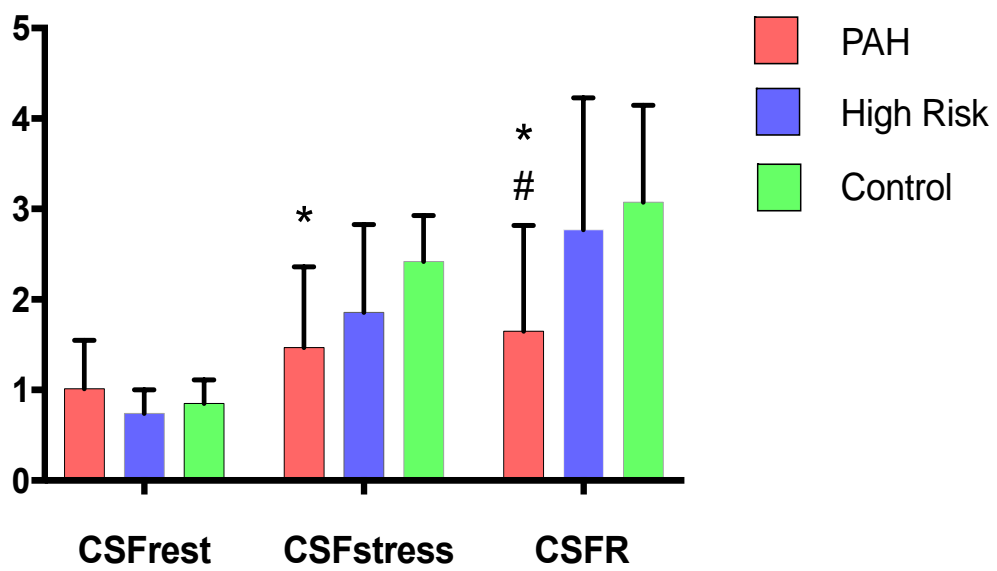


Figure 2: Differences in coronary sinus flow [CSF, ml/mg/min] measured at rest [CSFrest] and during adenosine-stress [CSFstress], and coronary sinus flow reserve [CSFR].

* P<0.05 PAH vs. Healthy Controls

P<0.05 PAH vs. High Risk

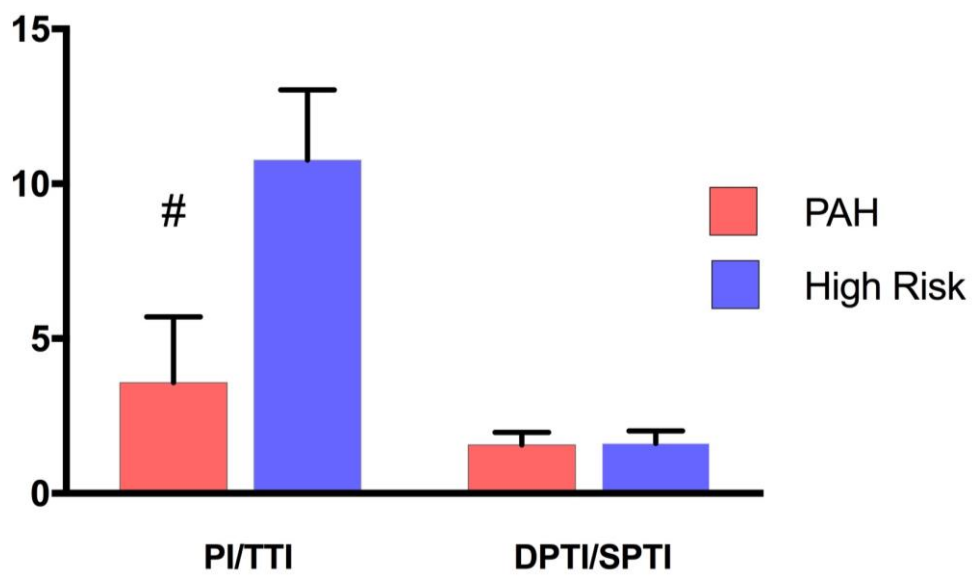


Figure 3: Differences in myocardial blood supply:demand indexes for the right (PI/TTI) and left (DPTI/SPTI) ventricles.

#P<0.0001 PAH vs. High Ris

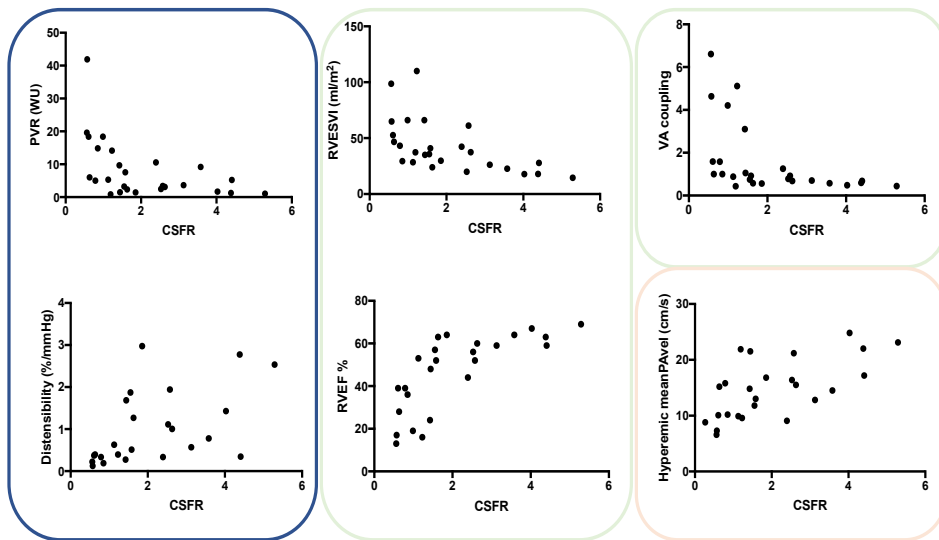


Figure 4: Scatterplots illustrating relationships between coronary sinus flow reserve (CSFR) with markers of RV afterload (blue), RV remodeling/performance (green), and cardiopulmonary reserve (orange).

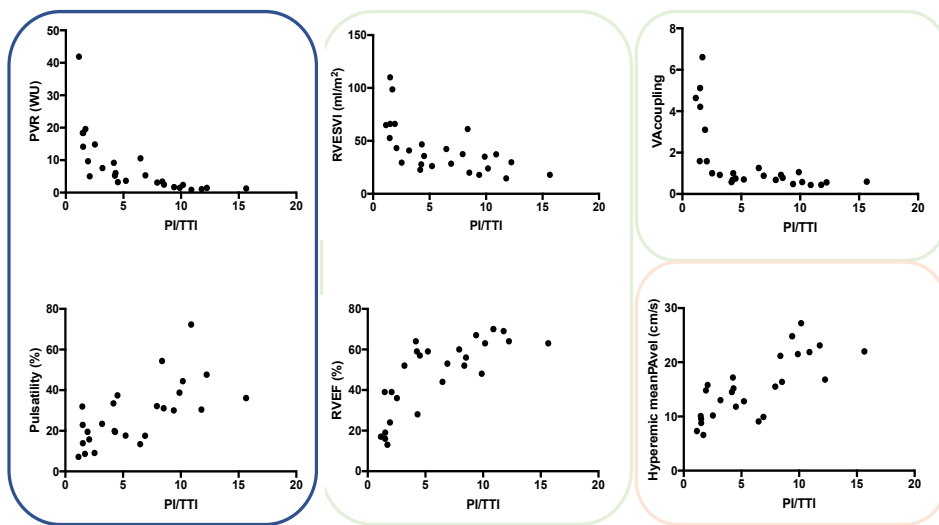


Figure 5: Scatterplots illustrating relationships between the myocardial coronary supply:demand index for the right ventricle (PI/TTI) and markers of RV afterload (blue), RV remodeling/performance (green), and cardiopulmonary reserve (orange).

TABLES

	PAH	High Risk	Healthy Controls	P value
Participants, n	17	9	9	
Female (%)	13 (76)	7 (78)	8 (89)	0.98
Age, years	54.3±14	60.2±14	46.9±12	0.13
Height, cm	162.6±12	164.1±9	165.6±7	0.75
Weight, kg	73.7±19	78±14	70.3±11	0.58
PAH etiology (%)				
Idiopathic	12 (71)			
Associated with CTD	5 (29)			
Comorbid conditions (%)				
Diabetes mellitus	4 (24)	0 (0)		<0.05
Connective tissue disease	5 (29)	6 (67)		<0.05
NYHA functional class (%)				
II	5 (29)	9 (100)		<0.05
III	12 (71)	0 (0)		<0.05
Therapy (%)				
ERA	1 (11)			
PDE5i	1 (11)			
Combination	7 (78)			
NTpBNP (pg/ml)	2203±1350	1555±824		0.29
Creatinine	81±21	75±34		0.58
Hemoglobin (g/L)	123±10	128±13		0.85
D _L CO (%)	52±17	61±15		0.3
%FVC/%D _L CO	1.6±0.88	1.4±0.18		0.48

Table 1: Demographic and clinical characteristics of participants.

CTD = connective tissue disease; NYHA = New York Heart Association; ERA = endothelin receptor antagonist; PDE5i = phosphodiesterase-5 inhibitor; NTpBNP = N-terminal pro brain natriuretic peptide; D_LCO = diffusing capacity for carbon monoxide; FVC = forced vital capacity.

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	Parameter	PAH	High Risk	P value	Correlation (R)	
					CSFR	PI/TTI
RV AL	mPAP (mmHg)	47±17	19 ± 4	<0.05	-0.65***	
	PVR (WU)	11.5±9.6	1.8 ± 0.8	<0.05	-0.59**	-0.93****
	Distensibility (%/mmHg)	0.5 ± 0.4	2.2 ± 1.1	<0.05	0.54**	
	Pulsatility (%)	20 ± 9	43 ± 14	<0.05	0.44*	0.66***
RV remodeling/ performance	RVmassI (g/m ²)	45 ± 19	28 ± 10	<0.05	-0.82****	-0.67***
	RAvoII (ml/ m ²)	85 ± 38	50 ± 22	<0.05	-0.69***	-0.57**
	RVEDVI (ml/ m ²)	88 ± 27	74± 32	0.25	-0.55**	-0.40*
	RVESVI (ml/ m ²)	49 ± 25	29 ± 15	<0.05	-0.72****	-0.70****
	RVEF (%)	40 ± 18	61 ± 8	<0.05	0.81****	0.80****
	VAcoupling	2.1 ± 1.9	0.7 ± 0.2	<0.05	-0.72****	-0.80****
Cardiopulmonary reserve	Hyperemic meanPAvel (cm/s)	12.2 ± 3.3	21.7 ± 3.4	<0.05	0.61**	0.79****
Myocardial supply:demand indexes	PI/TTI	3.6 ± 2.1	10.8 ± 2.3	<0.05	0.59**	
	DPTI/SPTI	1.6 ± 0.4	1.6 ± 0.4	0.79	-0.13	

Table 2: Absolute values of comparator parameters, and correlation coefficients with CSFR and PI/TTI.

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*P<0.05

**P<0.01

***P<0.001

****P<0.0001

mPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; RVmassI = indexed RV myocardial mass; RAvolI = indexed right atrial volume; RVEDVI/RVESVI = indexed RV end diastolic/systolic volumes; RVEF = RV ejection fraction

CHAPTER 6

CONCLUSION AND FUTURE DIRECTIONS

It is peculiar that pulmonary arterial hypertension - which has long been recognized as a “plexogenic pulmonary arteriopathy” [233] - continues to be defined by arbitrary, late pathophysiologic sequelae (i.e. pre-capillary pulmonary hypertension), particularly since certain populations at risk are readily identifiable (e.g systemic sclerosis and other connective tissue diseases), and contemporary, disease-modifying medical treatments are most effective when instituted early in the disease course. Furthermore, unlike left ventricular systolic dysfunction, there is a distinct lack of therapies specifically targeting the inevitable maladaptive right ventricular remodeling and systolic dysfunction that ensues. We designed and tested a novel, noninvasive pulmonary vasoreactive challenge utilizing cardiac magnetic resonance imaging and intravenous adenosine to: (i) determine feasibility, safety, and tolerability of this novel method to interrogate the right ventricular-pulmonary arterial unit (cardiopulmonary unit); (ii) evaluate the capacity of phase-contrast CMR parameters to provide surrogate measures of pulmonary haemodynamics at rest and during vasoreactive challenge; (iii) explore whether CMR-derived surrogate measures provide insight into the reserve of the cardiopulmonary unit across a spectrum of clinical risk phenotypes, thus providing proof-of-concept for the detection of early pulmonary vascular disease; (iv) assess the capacity to provide longitudinal prognostic insight; and, (v) explore the relationship between RV coronary perfusion, maladaptive RV remodeling, systolic dysfunction, and cardiopulmonary reserve. Key findings of this body of work are:

- This protocol, utilizing intravenous adenosine infusion (70-, 140-, 210mcg/kg/min; 3-minutes at each dose) and CMR measurement of mean pulmonary arterial blood flow velocity (meanPAvel) is safe, feasible and tolerable.
- MeanPAvel correlates closely with relevant invasive pulmonary haemodynamic measurements at rest and during all doses of adenosine infusion, suggesting it is a suitable non-invasive surrogate measure. Pulsatility correlates with resting pulmonary haemodynamics, but correlation weakens during adenosine infusion.
- Participants with suspected but excluded PAH (labelled as high risk for incident PAH – or possible early PVD – for the purposes of this study) have significantly greater haemodynamic responses to intravenous adenosine compared to participants with PAH.
- MeanPAvel measured during adenosine-induced hyperaemia is an excellent functional correlate for cardiopulmonary reserve (as it pertains to PVD) across a spectrum of clinical risk phenotypes as determined by traditional clinical, haemodynamic and biomarker parameters, as well as by vasoreactive haemodynamic changes.
- Diagnostic performance of meanPAvel at peak hyperaemia is excellent for the discrimination of participants with PAH, high risk for incident PAH, and healthy volunteers.
- MeanPAvel at peak hyperaemia correlates with validated clinical, biomarker, CMR-derived, and RHC-derived prognostic parameters at initial assessment and longitudinally over time.

- Right ventricular coronary perfusion parameters (myocardial supply:demand index and hyperemic coronary flow reserve) are impaired in PAH and directly related to RV remodelling, RV performance, RV afterload, and cardiopulmonary reserve

Acknowledging that validation studies are necessary for any new diagnostic/assessment tool, these findings have several potential clinical implications. It is widely accepted that prognosis in PAH remains unacceptably poor despite therapeutic advances over recent decades. Identification of PVD prior to a measureable rise in pulmonary pressures represents an important - but thus far elusive – strategy to improve clinical outcomes with available therapies. Detecting a depleted cardiopulmonary reserve by assessing pulmonary haemodynamic changes during exercise has been widely evaluated without reliable success, in part due to difficulties standardizing and performing exercise pulmonary haemodynamic tests, understanding the bounds of ‘normal’ physiology, and acquiring measurements via non-invasive means. Our studies suggest that meanPAvel can be reliably and reproducibly measured non-invasively by CMR, both at rest and during standardized adenosine-stress, overcoming some of the limitations of exercise stress testing. Furthermore, meanPAvel at peak hyperaemia performed excellently as a functional correlate for cardiopulmonary reserve in participants representing a spectrum of clinical risk phenotypes, providing proof-of-concept for the detection of PVD before the vascular bed is overwhelmed. Since current therapies mitigate the natural history of PAH, this would be an opportune time to initiate treatment. In established PAH, predicting and monitoring treatment response remains difficult despite frequent, comprehensive re-evaluation in expert PAH centres, which may in part reflect a reliance upon static, resting measures of the state of the RV-PA circuit. Simple, non-invasive, reproducible, quantitative measures of cardiopulmonary reserve should afford a dynamic – and more informative - assessment of the RV-PA unit, facilitating more timely

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treatment decision-making (e.g. transition to intravenous prostanoid therapy or referral for lung transplantation assessment). MeanPAvel at hyperaemia, and its change over time, correlated with validated prognostic markers in the present study, but larger trials with long-term follow up are necessary to determine whether independent and/or superior prognostic information is provided by this parameter. Finally, progression to RV failure and premature death seems inevitable without lung transplantation. We demonstrate a close relationship between RV coronary perfusion, RV maladaptive remodelling, systolic dysfunction, and uncoupling of the RV-PA circuit. In addition to lowering PVR with 'standard' PAH therapies, directly targeting determinants of RV coronary perfusion (e.g. slowing heart rate with ivabradine) may support RV performance (and, therefore, overall cardiac performance) deeper into the disease course, thereby delaying the major cause of death, RV pump failure.

FUTURE DIRECTIONS

The presented findings are encouraging and supported by sound rationale, but represent only the first step towards the provision of a novel, clinically available diagnostic and assessment tool. CMR is commonly used in established PAH but its application towards detection of early PVD is hindered by its relatively high cost and lack of availability compared to, for example, trans-thoracic echocardiography (TTE). Additionally, while our protocol was safe, feasible, and tolerable, side-effects related to central effects of adenosine (particularly at 210mcg/kg/min) were common. Modification of the protocol to address these limitations may facilitate larger validation trials that are the logical 'next step'. Further research should address:

1. Modification/adaptation of existing protocol

- An alternative adenosine infusion regimen may yield similar diagnostic performance, but improved tolerance (e.g. 50-, 100-, 150mcg/kg/min). We

demonstrated discrepant dose-dependent increases in meanPAvel between the three participant groups during IV adenosine. While between-group differences (and diagnostic performance) was greatest at peak hyperaemia, diagnostic performance was good at 140mcg/kg/min (AUC 0.98 PAH vs. High Risk; AUC 0.94 High Risk vs. Healthy Controls). Measuring meanPAvel at rest and maximal hyperaemia only (i.e. rest and after 2-minutes infusion at 210mcg/kg/min) may be a feasible alternative.

- TTE may be capable of measuring meanPAvel which could be investigated by a comparative study (same participants undergoing both CMR and TTE). TTE-derived meanPAvel may be measured by:
 - acquiring a Pulsed Wave Doppler (PW) sample 1.5-2cm distal to the pulmonic valve in the right ventricular outflow view of the parasternal short axis window;
 - tracing systolic and diastolic Doppler profiles to yield the meanPAvel across the cardiac cycle.
 - CMR measurement of meanPAvel removes the assumption that flow is uniform throughout the PA – this may be a limitation of TTE-derived meanPAvel.

2. *Validation of findings and derivation of appropriate cut-offs*

- Larger studies with long-term clinical follow-up are necessary to validate our findings. Specifically, the assumption that our High Risk group represented possible pre-clinical PVD can only be validated by such follow-up. This may best be achieved by a registry-type study where patients at risk of incident PAH (e.g. systemic sclerosis) undergo routine measurement of meanPAvel at hyperaemia.

With sufficient incident PAH cases, derivation of ‘at-risk’ or ‘early PVD’ cut-off values should be possible, enabling prospective validation studies.

- Patients with left ventricular diastolic dysfunction were excluded from our studies. It would be important (and interesting) to determine the impact of latent or overt heart failure preserved ejection fraction on meanPAvel at hyperaemia, and assess whether there is significant variation between pre- and post-capillary diseases.
- Reproducing and confirming the prognostic role of meanPAvel at hyperaemia in established PAH should be more feasible. With a larger sample size, the independent prognostic relevance and cut-off values at baseline and longitudinally over time could be evaluated. This could be conducted as a standalone prospective study, part of a prospective therapeutic trial, or as a registry-type study.

3. *Comparison with alternative, validated measures of cardiopulmonary reserve in PAH*

- Non-invasive cardiopulmonary exercise testing (CPET) is usually performed using an incremental ramp exercise protocol to maximum exercise capacity. While protocol standardization is lacking for PAH, typical exercise responses are described and peak oxygen uptake (VO₂) provides prognostically-relevant information that can assist with therapeutic decision making. Correlation between meanPAvel at hyperaemia and peak VO₂ (or other relevant CPET-derived parameters) in PAH patients would support the pathobiologic rationale for meanPAvel at hyperaemia performing as a functional correlate for cardiopulmonary reserve, as outlined in previous Chapters.

4. *Further investigation of the relationship between RV myocardial perfusion and RV failure*

- As discussed in Chapter 5, pre-clinical studies (with the capacity to manually manipulate physiologic parameters) clearly support RV ischemia as a cause –

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and potential treatment target - of RV failure when afterload is elevated. Our findings support these relationships in the clinical setting, but do not permit causation be assessed. The independent prognostic relevance of myocardial oxygen supply:demand index (PI/TTI) could be readily interrogated using retrospective, registry data since it is calculated using routine haemodynamic parameters. With validation, serial measurement of parameters that influence RV coronary perfusion may provide additional markers of disease progression and treatment response in ambulatory PAH patients, as well as providing new surrogate end-points for relevant therapeutic trials (e.g. medications specifically targeting these parameters).

In summary, this body of work comprises the design and rationale of a novel, non-invasive approach to the interrogation of the cardiopulmonary unit in the context of pulmonary vascular disease, and findings relating to the application of this protocol towards detection of early PVD, monitoring disease progression and treatment response in established PAH, and elucidating the relationship between right ventricular coronary perfusion, RV performance, and cardiopulmonary reserve. Further research is urgently required to develop a thorough understanding of the PVD pathophenotype at all disease stages which, if available, may enable substantial improvements in morbidity and mortality using currently available therapies.

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