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Cardiac magnetic resonance imaging in the diagnosis and determination of outcome of pulmonary hypertension

A thesis by

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BSc (MedSci) Hons, MBChB, MRCP

Conducted in the Scottish Pulmonary Vascular Unit,
Institute for Cardiovascular and Medical Sciences,
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Submitted for the degree of Doctor of Medicine

to

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Declaration

The work reported in this thesis was undertaken during my position as a Clinical Research Fellow at the Scottish Pulmonary Vascular Unit, Golden Jubilee National Hospital. I declare that, except where explicit reference is made to the contribution of colleagues who are formally acknowledged overleaf, that this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed Name _____

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This thesis is dedicated to my wife, Sarah, and our beautiful son, Samuel.

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List of Abbreviations

ALM	Area Length Method
AUC	Area Under The Curve
BMI	Body Mass Index
BNP	B-type Natriuretic Peptide
BSA	Body Surface Area
CCB	Calcium Channel Blocker
cGMP	Cyclic Guanosine Monophosphate
CHD	Congenital Heart Disease
CI	Cardiac Index
CMR	Cardiovascular Magnetic Resonance
CO	Cardiac Output
COPD	Chronic Obstructive Pulmonary Disease
CPET	Cardiopulmonary Exercise Testing
CT	Computed Tomography
CTPA	Computed Tomography Pulmonary Angiogram
CTD-PAH	Connective Tissue Disease Associated Pulmonary Arterial Hypertension
CTEPH	Chronic Thromboembolic Pulmonary Hypertension
CXR	Chest Radiograph
DPG	Diastolic Pulmonary Gradient
ECG	Electrocardiogram
ED	End-Diastole
EDV	End-Diastolic Volume
EDVI	End-Diastolic Volume Index
EF	Ejection Fraction

ES	End-Systole
ESV	End-Systolic Volume
ESVI	End-Systolic Volume Index
FEV ₁	Forced Expiratory Volume in 1 Second
FVC	Forced Vital Capacity
FISP	Fast Imaging With Steady State Precession
FLASH	Fast Low Angle Shot
HFpEF	Heart Failure With Preserved Ejection Fraction
HFrEF	Heart Failure With Reduced Ejection Fraction
HLA	Horizontal Long Axis
HR	Heart Rate
IGR	Inert Gas Rebreathing
IPAH	Idiopathic Pulmonary Arterial Hypertension
IVS	Interventricular Septum
LA	Left Atrium
LAV	Left Atrial Volume
LV	Left Ventricle/Ventricular
LVEDV	Left Ventricular End-Diastolic Volume
LVEDVI	Left Ventricular End-Diastolic Volume Index
LVEF	Left Ventricular Ejection Fraction
LVESV	Left Ventricular End-Systolic Volume
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
MCTD	Mixed Connective Tissue Disease
mPAP	Mean Pulmonary Artery Pressure
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging

MVO ₂	Mixed Venous Oxygen Saturation
NIH	National Institutes of Health
NO	Nitric Oxide
NT-proBNP	N terminal pro B-type Natriuretic Peptide
NYHA	New York Heart Association
PAH	Pulmonary Arterial Hypertension
PAP	Pulmonary Artery Pressure
PASP	Pulmonary Artery Systolic Pressure
PAWP	Pulmonary Artery Wedge Pressure
PBF	Pulmonary Blood Flow
PDE-5	Phosphodiesterase Type-5
PH	Pulmonary Hypertension
PVOD	Pulmonary Veno-Occlusive Disease
PVR	Pulmonary Vascular Resistance
RA	Rheumatoid Arthritis
RAP	Right Atrial Pressure
RAV	Right Atrial Volume
REVEAL	Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management
RHC	Right Heart Catheterisation
ROC	Receiver Operating Characteristics Curve
RV	Right Ventricle/Ventricular
RVEDV	Right Ventricular End-Diastolic Volume
RVEDVI	Right Ventricular End-Diastolic Volume Index
RVEF	Right Ventricular Ejection Fraction
RVESV	Right Ventricular End-Systolic Volume
RVM	Right Ventricular Mass

RVMI	Right Ventricular Mass Index
RVSV	Right Ventricular Stroke Volume
RVSVI	Right Ventricular Stroke Volume Index
SA	Short Axis
SD	Standard Deviation
sGC	Soluble Guanylate Cyclase
SLE	Systemic Lupus Erythematosus
SPVU	Scottish Pulmonary Vascular Unit
SSc	Systemic Sclerosis
SSFP	Steady State Free Precession
SV	Stroke Volume
SVI	Stroke Volume Index
T	Tesla
TAPSE	Tricuspid Annular Plane Systolic Excursion
TLC	Total Lung Capacity
TLCO	Transfer Factor for the Lung for Carbon Monoxide
TPG	Transpulmonary Gradient
TRPG	Tricuspid Regurgitation Peak Gradient
TTCW	Time to Clinical Worsening
UK	United Kingdom
VLA	Vertical Long Axis
VMI	Ventricular Mass Index
VO ₂	Oxygen Uptake
V/Q	Ventilation to Perfusion Ratio
WHO FC	World Health Organisation Functional Class
6MWD	Six Minute Walk Distance
6MWT	Six Minute Walk Test

% predicted 6MWD	percent predicted 6MWD
r	Pearson correlation coefficient
Δ	change
n	number

List of Publications

Scientific Papers

1. Crawley SF, Johnson MK, Dargie HJ, Peacock AJ. LA volume by CMR distinguishes idiopathic from pulmonary hypertension due to HFpEF. *JACC - Cardiovascular Imaging* 2013;6:1120-1121
2. Peacock AJ, Crawley SF, McLure LER, Blyth KJ, Vizza CD, Poscia R, Francone M, Iacucci I, Olschewski H, Kovacs G, Noordegraaf AV, Marcus JT, van de Veerdonk MC, Oosterveer FPT. Changes in right ventricular function measured by cardiac magnetic resonance imaging in patients receiving pulmonary arterial hypertension-targeted therapy the EURO-MR study. *Circulation - Cardiovascular Imaging* 2014;7:107-114

Abstracts

1. Crawley SF, Johnson MK, Peacock AJ. The changing face of pulmonary hypertension: The role of heart and lung disease. *Thorax*. 2010;65:A89 (poster presentation)
2. Crawley SF, Blyth KG, McLure LE, Dargie HJ, Peacock AJ. Prognostic value of right ventricular mass and function in connective tissue disease-associated pulmonary arterial hypertension. European Respiratory Society Annual Congress 2010:P1147 (poster presentation).

3. Crawley SF, Johnson MK, Peacock AJ. Improvement in right ventricular function following disease-targeted therapy for pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2011;183 (poster presentation)
4. Crawley SF, Johnson MK, Dargie HJ, Peacock AJ. Left atrial volume to distinguish idiopathic pulmonary arterial hypertension from pulmonary hypertension due to left heart disease. European Respiratory Society Annual Congress 2011:P4912 (oral presentation).
5. Crawley SF, Blyth KG, McLure LE, Dargie HJ, Peacock AJ. Left ventricular dysfunction influences survival in connective tissue disease associated pulmonary arterial hypertension but not idiopathic pulmonary arterial hypertension. *Thorax*. 2014;69:A64 (oral presentation)

Abstract

Pulmonary hypertension (PH) is a rare but serious condition that causes progressive right ventricular (RV) failure and death. PH may be idiopathic, associated with underlying connective-tissue disease or hypoxic lung disease, and is also increasingly being observed in the setting of heart failure with preserved ejection fraction (HFpEF). The management of PH has been revolutionised by the recent development of new disease-targeted therapies which are beneficial in pulmonary arterial hypertension (PAH), but can be potentially harmful in PH due to left heart disease, so accurate diagnosis and classification of patients is essential. These PAH therapies improve exercise capacity and pulmonary haemodynamics, but their overall effect on the right ventricle remains unclear. Current practice in the UK is to assess treatment response with 6-minute walk test and NYHA functional class, neither of which truly reflects RV function. Cardiac magnetic resonance (CMR) imaging has been established as the gold standard for the evaluation of right ventricular structure and function, but it also allows a non-invasive and accurate study of the left heart. The aims of this thesis were to investigate the use of CMR in the diagnosis of PH, in the assessment of treatment response, and in predicting survival in idiopathic and connective-tissue disease associated PAH. In Chapter 3, a left atrial volume (LAV) threshold of 43 ml/m^2 measured with CMR was able to distinguish idiopathic PAH from PH due to HFpEF (sensitivity 97%, specificity 100%). In Chapter 4, disease-targeted PAH therapy resulted in significant improvements in RV and left ventricular ejection fraction ($p < 0.001$ and $p = 0.0007$, respectively), RV stroke volume index ($p < 0.0001$), and left ventricular end-diastolic volume index ($p = 0.0015$). These corresponded to observed improvements in functional class and exercise capacity, although correlation coefficients between Δ 6MWD and Δ RVEF or Δ LVEDV were low. Finally, in Chapter 5, one-year and three-year survival was worse in CTD-PAH (75% and 53%) than in IPAH (83% and 74%), despite similar baseline clinical characteristics, lung function, pulmonary haemodynamics and treatment. Baseline right ventricular stroke volume index was an independent predictor of survival in both conditions. The presence of LV systolic dysfunction was of prognostic significance in CTD-PAH but not IPAH, and a higher LAV was observed in CTD-PAH suggesting a potential contribution from LV diastolic dysfunction in this group.

Chapter 1

Introduction

1. Introduction

Pulmonary hypertension (PH), defined as a resting mean pulmonary artery pressure (mPAP) ≥ 25 mmHg measured at right heart catheterisation, results from a variety of conditions that can affect the pulmonary circulation. There is a progressive increase in pulmonary vascular resistance (PVR), from either obstruction or obliteration of the pulmonary vascular bed, which causes right ventricular failure and premature death. In the prospective National Institutes of Health (NIH) study of 198 patients with PH conducted in the United States in 1980s, an era where there was no effective pharmacological therapy, median survival was only 2.8 years¹. Thankfully the last two decades have seen a substantial increase in our understanding of PH pathophysiology, and this has facilitated the development of several different classes of disease-targeted therapy. These new therapeutic options, initially aimed at pulmonary arterial hypertension (PAH) but now being increasingly used in PH due to other conditions, have resulted in significant improvements in survival. However, despite this progress PH remains an incurable condition, and is still associated with significant morbidity and mortality².

This chapter will discuss the background and rationale of the work undertaken in this thesis. It will review the current PH classification, pathology and pathobiology of PH, and the normal and abnormal right ventricle. Following this is a discussion on the diagnostic assessment of PH, with particular focus on the role of cardiac magnetic resonance (CMR) imaging, current therapeutic options and the changing demographic profile in PH. Finally, the aims and hypothesis of this thesis are introduced.

1.1 Classification of Pulmonary Hypertension

Pulmonary Hypertension was previously classified into two categories: primary pulmonary hypertension (where there was no underlying cause for the PH), and secondary pulmonary hypertension (where an underlying risk factor or cause existed).

In 1998 a new five-group clinical classification was proposed at the second World Symposium on Pulmonary Hypertension (Evian, France) which established individual PH groups based on similar clinical features, haemodynamics, pathological findings and therapeutic strategies. The five groups identified were pulmonary arterial hypertension

(Group 1); pulmonary hypertension due to left heart disease (Group 2); pulmonary hypertension due to chronic lung disease and/or hypoxia (Group 3); chronic thromboembolic pulmonary hypertension (Group 4); and pulmonary hypertension due to unclear pathological mechanisms (Group 5).

The Evian classification is now well established, but has been further refined at subsequent World Symposium(s) in Venice, Italy (2003), Dana Point, USA (2008), Nice, France (2013)³⁻⁵. At the most recent World Symposium the consensus was reached that the current classification should be maintained, with only minimal modifications to Group 1.

The Nice clinical classification is summarized in Table 1.1

Table 1.1 Clinical classification of pulmonary hypertension
(Nice Classification 2013)

<p>1 Pulmonary arterial hypertension</p> <p>1.1 Idiopathic PAH</p> <p>1.2 Heritable PAH</p> <p>1.2.1 BMPR2</p> <p>1.2.2 ALK-1, ENG, SMAD9, CAV1, KCNK3</p> <p>1.2.3 Unknown</p> <p>1.3 Drug and toxin induced</p> <p>1.4 Associated with:</p> <p>1.4.1 Connective tissue disease</p> <p>1.4.2 HIV Infection</p> <p>1.4.3 Portal hypertension</p> <p>1.4.4 Congenital heart diseases</p> <p>1.4.5 Schistosomiasis</p> <p>1' Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis</p> <p>1'' Persistent pulmonary hypertension of the newborn</p>
<p>2. Pulmonary hypertension due to left heart disease</p> <p>2.1 Left ventricular systolic dysfunction</p> <p>2.2 Left ventricular diastolic dysfunction</p> <p>2.3 Valvular disease</p> <p>2.4 Congenital / acquired left heart inflow / outflow tract obstruction and congenital cardiomyopathies</p>
<p>3. Pulmonary hypertension due to lung diseases and/or hypoxia</p> <p>3.1 Chronic obstructive pulmonary disease</p> <p>3.2 Interstitial lung disease</p> <p>3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern</p> <p>3.4 Sleep-disordered breathing</p> <p>3.5 Alveolar hypoventilation disorders</p> <p>3.6 Chronic exposure to high altitude</p> <p>3.7 Developmental lung diseases</p>
<p>4. Chronic thromboembolic pulmonary hypertension (CTEPH)</p>
<p>5. Pulmonary hypertension with unclear multifactorial mechanisms</p>

5.1 Haematologic disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy

5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphogioleiomyomatosis

5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders

5.4 Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

BMPR = bone morphogenic protein receptor type II; CAV1 = caveolin-1; ENG = endoglin; HIV = human immunodeficiency virus; PAH = pulmonary arterial hypertension

1.2 Pathology and Pathobiology of Pulmonary Hypertension

In WHO Group 1 PAH, pathological lesions typically affect distal pulmonary arteries of < 500µm diameter, with pulmonary veins usually unaffected^{6, 7}. The characteristic histopathological abnormalities in PAH are medial hypertrophy, fibrotic and proliferative intimal change, and thickening of the adventitia with moderate perivascular inflammatory infiltrates. These changes result in luminal occlusion (pulmonary vascular remodelling), the formation of angioproliferative 'plexiform' lesions at pulmonary artery bifurcation sites, and in-situ thrombus formation.

Different mechanisms contribute to the increased PVR observed in PAH, including vasoconstriction, proliferative and obstructive remodelling of the pulmonary vessel wall, inflammation, and thrombosis. The exact trigger for these pathological changes is unclear, but it is felt to be a multifactorial process involving several different biochemical pathways and cell types⁸⁻¹⁰. Endothelial dysfunction leads to overexpression of vasoconstrictor and proliferative substances such as thromboxane A2 and endothelin-1, and impaired production of vasodilator and antiproliferative agents such as nitric oxide and prostacyclin. Excessive vasoconstriction has been related to abnormalities of potassium channels in the smooth muscle cells. Within the adventitia, there is increased production of extracellular matrix including collagen, elastin, tenascin and fibronectin. PAH is a prothrombotic state, where thrombi can be present in both the small distal pulmonary arteries and the proximal elastic pulmonary arteries, further reducing lumen calibre. Other cell types that have been implicated in PAH include inflammatory cells, fibroblasts and platelets, and plasma mediators such as vasoactive intestinal peptide.

The pathological lesions observed in chronic thromboembolic PH (CTEPH) are characterised by organised thrombi that cause thickening of the vessel wall and luminal occlusion in proximal pulmonary arteries. They can also result in different grades of webs, bands and stenosis within elastic pulmonary arteries¹¹. In addition, a PAH-like arteriopathy can also affect occluded and non-occluded subsegmental pulmonary arteries. When CTEPH occurs following acute pulmonary embolism, non-resolution of the thromboembolic mass causes mechanical obstruction and pulmonary vascular remodelling, with increased shear force, pressure elevation and endothelial dysfunction in both occluded and non-occluded areas. It is recognized that at least 25% of patients with CTEPH do not have a history of acute pulmonary embolism, with pulmonary vascular remodelling in

these patients thought to be triggered by thrombotic or inflammatory lesions within a procoagulant environment^{12, 13}.

1.3 Right ventricular structure and function

The right ventricle (RV) was previously thought to be simply a conduit to transfer systemic venous blood to the lungs, and that the cardiovascular system could operate adequately without RV contractile function¹⁴. However, it has since become clear that the congenital RV hypoplasia can result in marked circulatory compromise and premature death. Advances in cardiovascular imaging with echocardiography and magnetic resonance have resulted in new opportunities to study RV structure and function, with particular interest in the pathophysiology of the RV in pulmonary hypertension.

The RV is the most anteriorly situated cardiac chamber, lying almost in the midline immediately behind the sternum. It has a complex shape, triangular when viewed from the side and crescent-shaped when viewed in cross section, unlike the ellipsoidal-shaped left ventricle (LV)¹⁵. In normal conditions, the interventricular septum (IVS) bows into the RV in both systole and diastole, due to LV pressures being higher than those in the RV and the subsequent positive left-to-right trans-septal pressure gradient.

The RV is often divided into three parts: (1) the inlet portion comprising the tricuspid valve, the papillary muscles and their associated chordae tendinae ; (2) the coarsely trabeculated apical myocardium; and (3) the outflow tract, also called the infundibulum or conus, consisting of smooth myocardium and the pulmonary valve¹⁶. The RV contains a prominent ridge of muscle known as the Moderator Band, which crosses the ventricular cavity from the apex to free wall, and is thought to assist in preventing over-distention of the RV.

The right and left ventricles are composed of multiple layers of muscle fibres which interlace to form a three-dimensional network¹⁶. The RV free wall is composed of superficial and deep muscle layers. In the superficial layer, the fibres are arranged circumferentially, in a direction parallel to the atrio-ventricular groove, and are anchored to the fibroskeleton of the heart around the tricuspid valve ring. These fibres turn obliquely near the cardiac apex, and continue into the superficial myofibres of the LV. The deep muscle fibres of the RV are aligned longitudinally from base to apex. In contrast, the LV is composed of obliquely oriented muscle fibres superficially, longitudinally oriented fibres in the deep (subendocardial) layer and intervening circumferential fibres.

Contraction of the RV commences at the inlet portion, and spreads sequentially to the trabeculated myocardium and then the infundibulum¹⁷. It is composed of three distinct mechanisms: (1) contraction of the circumferential fibres causes inward movement of the RV free wall; (2) shortening of the longitudinal fibres which draws the tricuspid annulus toward the apex; and (3) traction on the RV free wall at the points of attachment to the contracting LV. In the RV there is greater longitudinal than circumferential shortening, and a much lower contribution from the twisting and rotational movements that occur in the LV. The synchronous mechanical interaction between the ventricles throughout the cardiac cycle, “ventricular interdependence”, is achieved by the continuity in the muscle fibres of the RV and LV¹⁷.

The blood supply to the right ventricle will depend on whether the coronary system is right-dominant (80% of population) or left-dominant (20%). In the right-dominant system the right coronary artery supplies most of the RV, with marginal branches supplying the lateral wall, and the posterior descending artery supplying the posterior wall and the inferoseptal region¹⁸. The anterior wall and anteroseptal region are usually supplied by branches of the left anterior descending artery. In the normal RV, blood flow within the proximal right coronary artery occurs during both systole and diastole, with predominantly diastolic flow beyond the RV marginal branches¹⁹. It is felt that the RV is less vulnerable to irreversible ischaemic damage due to the combination of its extensive collateral system, lower oxygen consumption and ability to increase oxygen extraction.

In normal conditions, the RV is a low pressure/volume pump that moves desaturated blood out into the pulmonary circulation via the pulmonary valve. Desaturated blood returns from the systemic circulation via the superior and inferior vena cavae and right atrium, crosses the tricuspid valve and enters the inlet region of the RV

Differences in the function of the normal RV and LV can be explained by differences in muscle mass, orientation of myocardial fibres and chamber geometry²⁰⁻²², and the key differences are presented in Table 1.2

Table 1.2 Comparison of right and left ventricular structure and function

	Right Ventricle	Left Ventricle
Shape	crescentic in cross-section; triangular from the side	elliptic
Structure	inflow region, trabeculated apical myocardium and infundibulum	inflow region and myocardium and outflow tract
Muscle fibre orientation	superficial: circumferential deep: longitudinal	superficial: oblique middle: circumferential deep: longitudinal
Contractile pattern	long axis shortening, inward movement of RV free wall	twisting, rotation, wall thickening
End-Diastolic Volume (ml/m²)	75 ± 13	66 ± 12
Ejection fraction, %	61 ± 7	67 ± 5
Mass (g/m²)	26 ± 5	87 ± 12
Wall thickness, mm	2 to 5	7 to 11
Ventricular pressure, (mmHg)	average 25/4	average 130/8
PVR vs SVR (dyne · s · cm⁻⁵)	70	1100
Compliance at end- diastole	higher	lower
Filling profiles	starts earlier and finishes later, lower filling velocities	starts later and finishes earlier, higher filling velocities
Resistance to Ischaemia	greater resistance to ischaemia	more susceptible to ischaemia
Adaptation to disease state	more sensitive to pressure overload	more sensitive to volume overload

Modified from Haddad et al. Right ventricular function in cardiovascular disease, Part I. Circulation. 2008;117:1436-1448

1.3.1 Right ventricular response to increased afterload

RV systolic function is a reflection of preload, contractility and afterload. RV preload represents the load on the RV that is present before contraction, and the Frank-Starling mechanism dictates that under normal physiological conditions an increase in RV preload will improve myocardial contraction. However, excessive RV volume loading outwith normal physiological limits can result in LV compression and global impairment of ventricular function through the mechanism of ventricular interdependence²³. Filling of the RV can be influenced by active and passive atrial characteristics, heart rate, intravascular volume status, ventricular relaxation and chamber compliance, and the degree of pericardial constraint²⁴.

RV afterload represents the load that must be overcome during RV ejection. Since resting pulmonary vascular resistance (PVR) is only one-fifteenth of normal systemic vascular resistance, the RV is able to eject the equivalent volume of blood per contraction as the LV at a far lower energy cost, and this low pressure environment within the pulmonary circulation helps to prevent fluid migrating into the interstitial space, providing optimal conditions for gas exchange. However, the disadvantage is that the RV is more vulnerable to changes in afterload than the LV, with small increases in PVR causing in a marked reduction in RV stroke volume^{23, 25}.

In pulmonary hypertension there is a progressive increase in pulmonary vascular resistance, and extent of right ventricular adaptation to this increased afterload will determine the clinical course and outcome of the patient. Right ventricular adaptation to disease is a complex and multi-factorial process. It can be influenced by the time of onset of the disease (paediatric vs adult-onset heart disease), the time course of the disease (acute or chronic), and the severity of the myocardial stress or injury. The right ventricle can be affected by volume overload or pressure overload, ischaemia, pericardial constraint, or intrinsic pericardial disease²⁶. Volume-overload conditions (e.g. atrial septal defect, tricuspid regurgitation, anomalous pulmonary venous drainage) can be tolerated for prolonged periods without significant impairment of RV systolic function, although do eventually lead to increased morbidity and mortality^{27, 28}. In contrast, pressure-overload conditions (left-sided heart failure, pulmonary embolism, other causes of PH, RV outflow tract obstruction) are poorly tolerated in adult patients and often lead to RV dilatation and failure²³. In an acute pressure-overload state such as pulmonary embolism, even a

previously normal RV may be unable to adapt to the rapid increase in afterload caused by the embolic burden, and rapid right ventricular failure can ensue²⁹.

When chronic pressure-overload occurs in the setting of idiopathic PAH, there is an initial adaptive response of RV myocardial hypertrophy followed by progressive contractile dysfunction³⁰. Dilatation of the RV then occurs to facilitate compensatory preload, and to maintain preserved stroke volume despite reduced ejection fraction. As RV dysfunction progresses there is rising filling pressures, diastolic dysfunction and cardiac output continues to deteriorate³¹. This can be exacerbated by tricuspid regurgitation due to annular dilatation and poor leaflet coaptation, whilst increased RV size and pressure can also impair LV diastolic function³². In congenital heart diseases with left-to-right shunting, there is early exposure of the RV to systemic pressures thus allowing the RV to undergo hypertrophic growth in parallel to the LV. When increased afterload eventually occurs due to pulmonary vasculopathy and consequent shunt reversal, the hypertrophied RV is able to function normally for many years, and this can explain why survival in PH due to congenital heart disease is much greater than in PH due to other aetiologies³³⁻³⁵. The effects of altered gene expression, neurohormonal and cytokine activation on the pattern of RV modelling are thought to explain why the same degree of PH can cause early or late RV failure in different patients³⁶.

The initial response of the RV to an increased afterload is concentric hypertrophy, and this is predominantly caused by increased protein synthesis and an increase in cardiomyocyte size through the addition of sarcomeres^{37, 38}. Protein synthesis occurs in response to increased stretch, with a contribution from autocrine, paracrine and neurohumoral influences. Adaptive RV hypertrophy cannot preserve cardiac output indefinitely, and there is eventually transition to RV dilatation and failure. Functional, structural or numerical changes in cardiomyocytes lead to a reduction in cardiac contractile force and the RV dilates. Whilst the precise explanation for this phenotypic change is unknown, the increased wall tension that results from RV dilatation increases myocardial oxygen demand and simultaneously decreases RV perfusion. Increased afterload results in the RV returning to a fetal gene expression pattern, with a shift from α - to β -myosin heavy chain expression, phosphodiesterase type-5 expression, and an increase in adrenergic receptors^{36, 39}.

Impaired RV systolic function results in a longer contraction time and subsequent interventricular mechanical asynchrony, with the RV still contracting as the LV enters

diastole⁴⁰⁻⁴². The result is a positive right to left trans-septal pressure gradient during early diastole which causes leftward bowing of the interventricular septum into the LV cavity, impairment of LV diastolic filling, and reduced cardiac output. Available RV filling time (i.e. diastolic duration) is severely shortened, with compromise of RV filling, RV stroke volume and subsequent LV filling⁴³. This vicious circle of worsening contractility, increasing dilatation and falling cardiac output can be accelerated by abnormal neurohormonal signaling, immune activation and inflammatory responses, and cardiomyocyte apoptosis^{38, 44, 45}.

The extent of right ventricular dysfunction beyond which recovery is unlikely to occur remains unknown. Improvements in RV function have been demonstrated after single lung transplantation for pulmonary hypertension⁴⁶. Following living-donor lobar lung transplantation for PAH, right ventricular function recovered early (by 8 weeks) whereas recovery of left ventricular function required 6 to 12 months⁴⁷. Resolution of right ventricular dysfunction has been demonstrated after thrombolytic therapy for acute pulmonary embolism⁴⁸, and after balloon pulmonary angioplasty⁴⁹ or pulmonary endarterectomy for CTEPH⁵⁰.

In PAH, right ventricular function is the most important determinant of survival, with the prognostic significance of variables reflecting RV function (e.g. cardiac index, right atrial pressure, VO₂ max) far exceeding that of mean pulmonary artery pressure^{1, 23, 51, 52}. This may reflect the fact that pulmonary artery pressure can often decrease as RV failure progresses. The presence of right heart failure prior to the initiation of PAH therapy is associated with poor survival⁵³.

1.4 Clinical Presentation of Pulmonary Arterial Hypertension

Most patients with PAH present with exertional dyspnoea, developing over months or years⁵⁴. This classical, although non-specific, symptom is thought to be due to the inability of the right heart to raise output on exertion. Chest pain, syncope and peripheral oedema are more common in advanced PAH, and indicate right ventricular failure.

The clinical signs of PAH, whilst subtle, can include right ventricular heave, loud pulmonary component of the second heart sound, a pansystolic murmur of tricuspid regurgitation and a right ventricular third sound. The lungs are typically clear. Jugular venous distension, hepatomegaly, peripheral oedema, ascites and cold extremities indicate patients in a more advanced state with right ventricular failure at rest. Central cyanosis may also be present in advanced cases.

Clinical examination may also help identify the aetiology of the pulmonary hypertension. In PAH due to scleroderma there may be digital ulceration, telangiectasia and sclerodactyly. In PAH associated with portal hypertension there may be stigmata of chronic liver disease, such as palmar erythema, spider naevi, gynaecomastia and testicular atrophy. PH due to underlying interstitial lung disease may be suggested by the presence of fine inspiratory crackles⁵⁵.

1.5 Diagnostic Assessment of Pulmonary Arterial Hypertension

The diagnostic assessment of a patient with suspected pulmonary arterial hypertension requires a series of investigations that will confirm the diagnosis, clarify the clinical group of PH (i.e. WHO Group 1-5) and the specific aetiology within the PAH (Group 1) group, and evaluate the functional and haemodynamic impairment.

The ECG may provide evidence of right ventricular hypertrophy or strain, with right axis deviation, large p-waves especially in lead II, tall R waves and ST depression in the right-sided chest leads. Right axis deviation is present on ECG in 79% of patients with idiopathic PAH, and RV hypertrophy is present in 87%⁵⁴. Most patients with PAH will be in sinus rhythm, however the presence of supraventricular arrhythmias such as atrial flutter can indicate advanced disease, and are often associated with rapid clinical deterioration⁵⁶. Atrial fibrillation is common in patients with pulmonary hypertension associated with left heart disease⁵⁷.

The chest X-ray will be abnormal in most patients with pulmonary arterial hypertension⁵⁴. Common findings include enlargement of the main pulmonary arteries and rapid tapering of the vessels as they extend to the periphery of the lungs, giving rise to peripheral oligoemia. Right ventricular and right atrial enlargement may also be visible in advanced disease. The chest X-ray can demonstrate upper lobe venous diversion and pleural effusions in pulmonary venous hypertension, and evidence of moderate-to-severe interstitial lung disease in Group 3 PH.

Pulmonary function tests (PFTs) in PAH often reveal normal or only mildly impaired spirometry, but a markedly reduced diffusion capacity for carbon monoxide. If clinically suspected, screening polysomnography or overnight oximetry should be used to exclude significant sleep-disordered breathing. A decrease in lung volume together with a decrease in diffusion capacity for carbon monoxide may indicate a diagnosis of interstitial lung disease, whereas non-reversible airflow obstruction together with increased residual volumes and reduced diffusion capacity for carbon monoxide suggests COPD as a cause of hypoxic pulmonary hypertension.

The key features of pulmonary arterial hypertension on CT are: enlargement of the main pulmonary artery (i.e. main pulmonary artery diameter >29mm)⁵⁸; right ventricular dilatation and hypertrophy, and enlargement of the right atrium; flattening or 'bowing' of

the interventricular septum; the presence of pericardial effusions; small centrilobular ground-glass nodules; reflux of contrast from the right atrium into the inferior vena cava when CT pulmonary angiography is performed. This last feature occurs as a result of tricuspid regurgitation and the extent of reflux correlates with mean pulmonary artery pressure, however it can be observed in normal patients⁵⁹.

High-resolution CT scanning can facilitate the diagnosis of emphysema and interstitial lung disease, and can be useful when the clinical history suggests possible pulmonary veno-occlusive disease (PVOD). Characteristic changes of PVOD include interstitial oedema with diffuse central ground-glass opacification and thickening of interlobular septa, whilst lymphadenopathy and pleural effusions may also be present.

The hallmark of CTEPH on CT pulmonary angiography is the absence or sudden loss of contrast filled vessels. Abnormalities in the main pulmonary artery may be highly suggestive of CTEPH, with the most common finding being the eccentric location of thrombus, resulting in a crescentic filling defect adjacent to the vessel wall⁶⁰. Bronchial artery hypertrophy occurs in approximately half the patients with CTEPH and only rarely in IPAH⁶¹. In CTEPH there is “mosaic oligoemia” visible on high resolution scanning, caused by obliteration of parts of the vascular bed. This results in hypoperfusion with arteries of diminished size in some areas, with normal or increased perfusion and enlarged arteries in others. Peripheral lung parenchymal opacities are another common finding in CTEPH⁶². They represent pulmonary infarcts due to occlusion of segmental and smaller pulmonary arteries and therefore occur more commonly in “peripheral type” CTEPH than “central type”.

Conventional pulmonary angiography is still used in some centres for the diagnosis and assessment of surgically correctable CTEPH. The typical findings include vascular cut-offs representing complete occlusion of the vessel, and vascular webs which result from organisation of thromboembolic material in the vessel lumen with subsequent scar formation. Smaller pulmonary arteries can appear tortuous and taper rapidly, particularly in patients with distal inoperable CTEPH. Magnetic resonance angiography does not require exposure to radiation, and is becoming increasingly important in the diagnosis, assessment and long-term follow-up of patients with CTEPH.

The role of ventilation perfusion (V/Q) scintigraphy is to distinguish IPAH from CTEPH. In IPAH, the VQ scan is usually normal or shows heterogeneous perfusion⁶³. Occasionally larger mismatch perfusion defects may be seen IPAH, thought to be as a result of thrombosis *in situ*. With CTEPH, the VQ scan is usually high probability, showing multiple mismatched segmental or larger perfusion defects. A normal or low probability scan effectively excludes CTEPH^{64, 65}. Whilst VQ scintigraphy is a safe and highly sensitive test for suspected CTEPH, large mismatch perfusion defects may arise in other processes that result in obliteration of the central arteries and veins. These “CTEPH mimics” include large vessel vasculitis, pulmonary artery sarcoma, extrinsic compression due to cancer, lymphadenopathy, fibrosing mediastinitis and pulmonary veno-occlusive disease (PVOD)⁶⁶.

Blood testing including routine haematology, biochemistry and thyroid function tests is performed in all patients. Serological testing is used to detect HIV, hepatitis and underlying connective-tissue disease, although up to 40% of patients with idiopathic PAH have elevated anti-nuclear antibodies, usually in low titre (1:80)⁶⁷. It is particularly important to exclude underlying systemic sclerosis (SSc) due to the poor survival associated with SSc-PAH. Anti-centromere antibodies are usually positive in limited scleroderma, and anticardiolipin antibodies may suggest a diagnosis of systemic lupus erythematosus. When a diagnosis of CTEPH is being considered then thrombophilia screening with anti-phospholipid antibodies, lupus anticoagulant, and anti-cardiolipin antibodies should be performed².

1.5.1 Echocardiography

The use of echocardiography in the assessment of pulmonary arterial hypertension focuses on detection of elevated pulmonary artery pressure, evaluation of the right ventricle and exclusion of other possible differential diagnosis.

In PAH chronic progressive pressure-loading results in right ventricular remodelling, particularly RV hypertrophy and later RV dilatation. RV dilatation can result in tricuspid annular dilatation, causing significant tricuspid regurgitation. Application of continuous wave Doppler mapping on this jet allows calculation of the TR velocity. This velocity represents the RV to right atrial pressure difference, and can be used to estimate pulmonary artery systolic pressure (PASP) through the Bernoulli equation:

$$\text{PASP} = \text{RV Systolic Pressure} = 4 (V_{\text{TR}})^2 + \text{RAP}$$

(V_{TR} = tricuspid regurgitant velocity, RAP = right atrial pressure)

Whilst calculation of mean PAP from PA systolic pressure is possible (mean PAP = 0.61 x PA systolic pressure + 2 mmHg), doppler echocardiography has been found to be inaccurate at assessment of pulmonary artery pressure in nearly 50% of cases⁶⁸. Use of the Bernoulli equation can lead to over- or under-estimations of PA systolic pressure by up to 10mmHg, and therefore pulmonary hypertension cannot be reliably defined by a cut-off value of Doppler-derived PA systolic pressure.

As PAH progresses there is impaired RV diastolic function, increased RV end-diastolic pressure, with displacement or “bowing” of the interventricular septum towards the left ventricle, and dilation of the main pulmonary artery.

Tricuspid annular plane systolic excursion (TAPSE) represents the basal to apical shortening of the right ventricle during systole. TAPSE will be reduced when there is impaired RV systolic function, and a TAPSE of less than 1.5cm is associated with poor prognosis in pulmonary arterial hypertension ($\leq 1.7\text{cm}$ in SSc-PAH)^{69, 70}. Other echocardiographic indices with prognostic significance include the presence of a pericardial effusion^{71, 72}, indexed right atrium area⁷² and the RV Doppler index^{73, 74}.

Echocardiography can also help identify the cause of suspected or confirmed PH. Doppler and contrast examinations can be used to detect underlying congenital heart disease. Pulmonary blood flow assessment and assessment of proximal pulmonary artery diameter can be used in the diagnosis of sinus venosus-type atrial septal defect or anomalous pulmonary venous drainage.

1.5.2 Exercise Capacity

The two commonly used methods for assessing exercise capacity in suspected or confirmed PAH are the 6-minute walk test (6MWT) and cardiopulmonary exercise testing (CPET).

The 6MWT is a non-invasive and reproducible assessment of exercise capacity. It is a self-paced test determining submaximal exercise capacity that is technically simple and inexpensive. Patients are asked to walk on a flat surface for 6 minutes, covering as much distance as possible at their own pace, with encouragement at 1-min intervals^{75, 76}. Total distance walked (6MWD), oxygen saturations and dyspnoea (Borg scale) are recorded. % predicted 6MWD can also be measured, adjusting the total distance walked for age, sex, height and weight, but its prognostic value was not superior to absolute 6MWD⁷⁷.

The 6 minute walk distance (6MWD) can also be affected by other factors including learning effect, patient motivation, general fitness, and coexisting musculoskeletal or rheumatological conditions. This can be of particular importance in patients with PAH associated with connective-tissue disease or hypoxic lung disease. The utility of the 6MWT may be limited in patients with milder or early pulmonary arterial hypertension who may have a relatively high baseline 6MWD (the 'ceiling' effect)^{78, 79}. In these patients PAH treatment may improve pulmonary haemodynamics and functional class, but not significantly alter 6MWD⁸⁰.

In idiopathic PAH, baseline 6MWD is predictive of survival and correlates with WHO functional class and haemodynamic parameters^{81, 82}. It has been demonstrated that after long-term epoprostenol therapy, absolute values of 6MWD > 380 m correlated with improved survival, whereas change in 6MWD from baseline did not⁵³. The 6MWT is widely used to assess response to PAH treatment, however it is yet to be demonstrated that changes in 6MWD after treatment correspond to improved survival. Recent meta-analysis

of PAH therapeutic trials found no relationship between 6MWD changes and clinical outcomes⁸³ or survival⁸⁴. An analysis of data from a recent clinical trial of the phosphodiesterase-5 inhibitor tadalafil has proposed that the minimal important difference in the 6MWT for PAH is approximately 33m, i.e. an improvement of 6MWD of +33m was associated with an improvement in quality of life measures⁸⁵.

During cardiopulmonary exercise testing, ventilation and gas exchange are continuously recorded throughout incremental exercise on a cycle ergometer or treadmill. Ventilation/perfusion mismatching and impaired oxygen transport can cause impaired exercise tolerance in PAH. At CPET this results in reduced peak oxygen pulse (VO_2/HR), reduced peak work rate, peak heart rate and ventilatory efficiency, and reduced oxygen uptake at anaerobic threshold and peak exercise. A peak $\text{VO}_2 < 10.4$ ml/min/kg and peak systolic blood pressure below 120 mmHg have been shown to be independent predictors of poor outcome in idiopathic PAH⁸⁶. Due to a lack of standardization and sufficient expertise performing CPET, the 6MWT remains, at present, the only Food and Drug Administration / European Agency for the Evaluation of Medicinal Products accepted exercise endpoint for studies evaluating treatment effects in PAH².

1.5.3 Inert Gas Rebreathing

The inert gas rebreathing (IGR) method allows pulmonary blood flow (PBF) to be measured during the rebreathing of a mixture of blood-soluble and blood-insoluble gases⁸⁷. When rebreathing through a respiratory apparatus from a bag prefilled with the gas mixture for about 30 seconds, the blood-soluble gas dissolves rapidly in the pulmonary capillary blood, and its rate of disappearance from the alveoli is in proportion to the effective pulmonary blood flow. The blood-insoluble gas is not taken up in the pulmonary capillary blood, and remains in the alveoli to allow for correction in total alveolar volume during the rebreathing manoeuvre. In the absence of significant intracardiac or intrapulmonary shunts then the measured PBF will equal cardiac output.

The recently developed metabolic IGR system called Innacor (Innovision, Odense, Germany) has been demonstrated to be a reliable and reproducible way of measuring cardiac output in patients with heart failure⁸⁸, interstitial lung disease⁸⁹, and pulmonary

hypertension⁹⁰. The Innocor device is portable, easy to maintain and can be used to provide measurements at rest and during exercise.

1.5.4 Natriuretic Peptides

Biochemical markers are a commonly used non-invasive tool for assessment and monitoring of RV dysfunction in patients with pulmonary hypertension. Brain natriuretic peptide (BNP) induces vasodilatation and natriuresis, and is released from myocardium in response to wall stress. The final step of BNP synthesis consists of a high molecular weight precursor, proBNP, cleaved into biologically inactive N-terminal segment (NT-proBNP) and the proper low molecular weight BNP⁹¹. NT-proBNP has a better stability both in circulating blood and after sampling due to its longer half-life.

NT-ProBNP has been shown to be an objective, non-invasive method for identifying right ventricular dysfunction in pulmonary hypertension^{92, 93}. In a heterogenous group of patients with chronic precapillary PH, plasma NT-proBNP was used to determine the clinical severity of disease and was independently associated with long-term mortality⁹⁴. In patients with systemic sclerosis an elevated NT-proBNP (>395 pg/ml) was associated with a very high probability of having PAH, and a 10-fold increase in NT-proBNP on therapy was associated with a greater than three-fold increase in mortality in SSc-PAH⁹⁵. Serial BNP/NT-proBNP measurements have been used to track RV function over 12 months in deteriorating or improving patients with PAH⁹⁶, and to identify residual PH following pulmonary endarterectomy for CTEPH⁹⁷.

1.5.5 Right Heart Catheterisation

Right heart catheterisation remains the gold standard for the diagnosis of pulmonary arterial hypertension, and is required to confirm the diagnosis of PAH, to assess the haemodynamic severity, to test the vasoreactivity of the pulmonary circulation, and to help select the appropriate therapy. Right heart catheterisation is performed using a Swan-Ganz thermodilution catheter, inserted into a central vein and floated through the right heart chambers into the pulmonary artery⁹⁸. The following variables are measured: right atrial pressure, right ventricular pressure, pulmonary artery pressure (systolic, diastolic and mean) and pulmonary artery occlusion pressure. Pulmonary vascular pressures are measured at end-expiration when the lungs are at functional residual capacity. Cardiac output (CO) is measured in triplicate by thermodilution or by the Fick method. Sampling of oxygen saturations from different sites can be used to identify any 'step-up' or 'step-down' in oxygen saturation to detect and quantify shunting.

Transpulmonary gradient (TPG) and pulmonary vascular resistance (PVR) are derived from the following equations:

$TPG = mPAP - \text{pulmonary artery occlusion pressure}$

$PVR = TPG/CO$, where

TPG = transpulmonary gradient

mPAP = mean pulmonary artery pressure

PVR = pulmonary vascular resistance

CO = cardiac output

The haemodynamic definitions of pulmonary hypertension are displayed in Table 1.3

Table 1.3 Haemodynamic definitions of pulmonary hypertension

Definition	Characteristics	Clinical Group(s) per Dana Point Classification
Pulmonary hypertension (PH)	Mean PAP \geq 25mmHg	All
Pre-capillary PH	Mean PAP \geq 25mmHg PAWP \leq 15mmHg CO normal or reduced*	1. Pulmonary arterial hypertension 3. PH due to lung diseases 4. Chronic thromboembolic PH 5. PH with unclear and/or multifactorial mechanisms
Post-capillary PH Passive Reactive	Mean PAP \geq 25mmHg PAWP $>$ 15mmHg CO normal or reduced TPG \leq 12 mmHg TPG $>$ 12 mmHg	2. PH due to left heart disease

All values to be measured at rest

* High CO can be present in cases of hyperkinetic conditions such as systemic-to-pulmonary shunts (only in the pulmonary circulation), anaemia, hyperthyroidism, etc.

CO = cardiac output; PAP = pulmonary arterial pressure; PH = pulmonary hypertension; PAWP = pulmonary artery wedge pressure; TPG = transpulmonary pressure gradient (mean PAP – mean PAWP)

Accurate recording of PAWP is essential for the diagnosis of PH due to left heart disease, particularly PH due to heart failure with preserved ejection fraction (HFpEF). It was traditionally felt that a PAWP > 15 mmHg effectively excluded the diagnosis of pre-capillary PAH, however there is growing recognition of the variability of wedge pressure measurements and the need for standardisation of methodology. A review of PAWP data from the REVEAL registry highlighted a number of cases where there was a significant difference in PAWP between initial and follow-up RHC⁹⁹. When there is a strong clinical suspicion of Group 2 PH, but initial PAWP is \leq 15mmHg, then fluid challenge with 500ml saline over 5-10 minutes has been used to “unmask occult pulmonary venous hypertension” by showing a disproportionate rise in PAWP¹⁰⁰. In some cases, left heart catheterisation may be required for direct assessment of LV end-diastolic pressure.

In PAH, vasoreactivity testing may be performed at the time of diagnostic RHC to identify patients who may benefit from long-term therapy with calcium channel blockers (CCBs). An acute vasodilator challenge is usually performed with inhaled nitric oxide, although intravenous epoprostenol or adenosine have also been used. A positive acute response is defined as a reduction of mPAP \geq 10 mmHg to reach an absolute value of mPAP \leq 40 mmHg with increased or unchanged cardiac output¹⁰¹. Approximately 10% of idiopathic PAH are acute responders and about half of these acute responders are also positive long term responders to calcium channel blockers. The role of vasoreactivity testing in non-idiopathic PAH (e.g PAH due to connective tissue disease or HIV) is less clear, and it is not recommended in PH Groups 2-5.

1.5.6 Complications associated with right heart catheterisation

Right heart catheterisation (RHC) is one of the most commonly performed procedures in current medical practice, with over 1 million performed in the USA annually¹⁰². The vast majority of RHCs are performed without incident, however complications may occur in association with central venous access, catheter insertion and catheter manipulation¹⁰³. These include accidental arterial puncture, arteriovenous fistula, air embolism, pneumothorax, cardiac arrhythmias, thrombosis and infection. Pulmonary artery rupture, which can occur following eccentric balloon inflation in small distal vessels, is associated with a 70% mortality rate but occurs in only 0.031% of procedures¹⁰⁴.

The potential hazards of performing RHC in patients with pulmonary hypertension have been a long-standing concern¹⁰⁵. Early reports had described high rates of serious adverse events and fatal complications^{54, 106}. A recent large multi-centre study addressing this issue found a serious adverse event rate of 1.1%¹⁰⁷. The most frequent complications were related to venous access (e.g. haematoma, pneumothorax), followed by arrhythmias and hypotensive episodes related to vagal reactions or pulmonary vasoreactivity testing. Overall procedure related mortality was low at only 0.055% (four deaths in 7218 procedures), however if RHC frequency could be reduced without reducing standard of care this would be an advantage for both patient safety and comfort.

1.6 Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance (CMR) imaging is a well established technique in the diagnosis and management of a wide range of cardiovascular diseases. Advances in CMR technology, with improved ECG gating and respiratory suppression, have facilitated rapid and robust data acquisition, so that now even very dyspnoeic patients can usually complete a CMR scan. It provides 3-dimensional anatomical measurements of cardiac morphology that are unaffected by coexisting conditions e.g. obesity, lung disease, heart rate, posture or hydration status. CMR imaging produces tomographic still images that accurately and reproducibly assess right and left ventricular chamber sizes, wall thickness and mass, and is used for both morphological and functional assessment.

Steady state free-precession pulse (SSFP) sequences are used to construct a cine image, a movie of 15–20 frames, in which the full cardiac cycle can be observed. The contrast between cavity blood and myocardium make planimetry of the interface accurate and easily reproducible for assessment of left and right ventricular function¹⁰⁸. Ventricular volumes and myocardial mass are typically obtained from a stack of contiguous “bright blood” cine CMR 5–10 mm slices covering the left and right ventricles acquired in short-axis or transverse orientation. Epicardial and endocardial contours are added during post-processing on end-diastolic and end-systolic frames, and right and left ventricular volumes are calculated as the sum of individual slice volumes¹⁰⁹. Left (LVESV) and right ventricular end-systolic volumes (RVESV) are subtracted from the end-diastolic volumes, giving stroke volumes. Ejection fraction represents stroke volume divided by end-diastolic volume. Ventricular mass is the product of myocardial volume and muscle-specific density (1.05 g/cm³). Analysis of cine data to produce ventricular volumes and masses was previously very time-consuming, however advances in computerized semi-automated myocardial/blood border definition has enabled analysis to become much faster.

1.6.1 Accuracy and Variability of Cardiac Magnetic Resonance Imaging

CMR is an accurate, precise and reliable method for measuring cardiac volumes, mass and function, in healthy individuals and in a variety of disease states.

In one of the earliest studies using CMR, ECG-gated MR images of the left ventricle were compared with X-ray contrast ventriculograms, with excellent correlation observed between the imaging modalities¹¹⁰. In a later study of hypertensive patients, CMR estimates of LV mass were within 17.5g (95% confidence interval) of the true LV mass at autopsy, and had superior precision and reliability compared with echocardiography¹¹¹. Subsequent work investigating LV dimensions and function have demonstrated that CMR has excellent interstudy reproducibility in normal, dilated, and hypertrophic hearts, superior to 2-dimensional echocardiography¹¹².

Grouthes *et al* demonstrated that whilst interstudy reproducibility values are lower for the complex-shaped right ventricle than for the left ventricle, CMR remains a reliable method for assessing the RV and is a suitable modality for serial RV assessment¹¹³. In a repeatability study of 10 PAH patients, manual analysis measures were found to be more robust than semiautomated analysis, and repeatability improved if the interventricular septum was wholly apportioned to the LV¹¹⁴.

1.6.2 Cardiac Magnetic Resonance Assessment of Pulmonary Hypertension

1.6.2.1 Ventricular volumes and function

In patients with pulmonary hypertension right ventricular dilatation results in increased CMR RV end-diastolic and end-systolic volumes compared with healthy volunteers¹¹⁵. There is reduced RV ejection fraction, lower RV stroke volume and poorer cardiac output in PH patients compared with normal individuals¹¹⁶.

CMR imaging has also demonstrated reduced LV end-diastolic volume, LV stroke volume and LV peak filling rate in patients with pulmonary hypertension compared with healthy controls^{42, 116}. A low LVEDV is felt to be the result of impaired LV filling, which can be due to the combination an elevated PVR limiting RV stroke volume and left ventricular septal bowing reducing LV volume in early diastole^{117, 118}. Gan *et al* demonstrated that leftward interventricular septum curvature correlated with left ventricular filling rate and left ventricular end-diastolic volume, concluding that ventricular interaction mediated by the interventricular septum impairs left ventricular filling, contributing to decreased stroke volume⁴².

In their study of 64 patients with IPAH assessed with CMR at diagnosis and after 1-year follow-up, Van Wolferen *et al* found that a low stroke volume ($\leq 25 \text{ ml/m}^2$), high RV end-diastolic volume ($\geq 84 \text{ ml/m}^2$) and low LV end-diastolic volume ($\leq 40 \text{ ml/m}^2$) were strong independent predictors of mortality and treatment failure¹¹⁸. A subsequent CMR study from the same group has shown that the minimal important difference (MID) of stroke volume after PAH therapy was 10ml, i.e. changes of $\geq 10\text{ml}$ should be considered clinically relevant¹¹⁹.

1.6.2.2 Ventricular Mass

In pulmonary hypertension, increased afterload results in right ventricular hypertrophy, with increased RV mass observed at CMR when compared with healthy controls^{120, 121}. There was no difference in LV mass between normal individuals and patients with IPAH^{120, 122}. Saba *et al* demonstrated that a ventricular mass index ((VMI), the ratio of right ventricular mass over left ventricular mass) of >0.6 , had a sensitivity of 84% and

specificity of 71% for detecting pulmonary hypertension of various aetiologies¹²². There was a strong correlation between VMI and mPAP ($r = 0.81$), with VMI having superior correlation to mPAP than right ventricular mass alone. In patients with systemic sclerosis undergoing diagnostic assessment, VMI correlated strongly with mPAP, and had prognostic value, with two-year survival in patients with VMI <0.7 and ≥ 0.7 of 91% and 43%, respectively¹²³. However, in the Van Wolferen study of patients with IPAH, RV hypertrophy (as measured by RV mass index) did not appear to be as strongly related to mortality as RV dilatation¹¹⁸.

1.6.2.3 Interventricular Septal Configuration

It is recognized that abnormal interventricular septal position and motion can occur in the setting of right ventricular pressure overload. In children with a range of congenital heart defects, echocardiography has demonstrated a leftward shift and flattening of the interventricular septum during systolic contraction, with marked exaggeration of this change occurring when RV systolic pressures were $>50\%$ of systemic pressure¹²⁴. Severe left ventricular septal bowing is often considered to be associated with an unfavourable prognosis in pulmonary hypertension, and has been associated with an adverse response to CCB trial in IPAH¹²⁵. Normalisation of septal abnormalities has been observed at echocardiography following bilateral lung transplantation for advanced IPAH¹²⁶, and at CMR after pulmonary endarterectomy for CTEPH¹²⁷. Using CMR and ventricular pressure measurements, Roeleveld *et al* found that maximal distortion of the normal septal shape occurred during right ventricular relaxation, occurring when pressure within the RV exceeded that within the LV¹²⁸. The degree of septal curvature was proportional to systolic PAP, with leftward septal bowing likely to occur when sPAP exceeded 67mmHg. By measuring both RV longitudinal shortening (apex-base distance change) and transverse shortening (septum-free wall distance change), it has been observed that declining RV function in advanced PAH is likely due to progressive leftward septal displacement with a decrease in transverse shortening¹²⁹. Contrast-enhanced CMR has also demonstrated delayed contrast enhancement, a well-established marker of myocardial abnormalities, present within the interventricular septum of PH patients with abnormal septal motion¹³⁰.

1.6.3. Estimation of pulmonary haemodynamics by cardiac magnetic resonance

There has been sustained interest in using non-invasive CMR as a method for estimating pulmonary haemodynamics in a variety of settings, including screening for PH, diagnosis, monitoring response to therapy and assessment of clinical deterioration¹³¹. In a group of 44 patients with PH, five different MRI-based estimators of PAP were compared with invasive pressure measurements, with VMI found to show the best correlation with mean PAP ($r = 0.56$, $p < 0.001$) but not accurate enough to replace right heart catheterisation in clinical practice¹³². A computed method that combines a number of CMR variables (including main pulmonary artery blood flow velocity at peak systole, maximal systolic main pulmonary artery cross sectional area) with biophysical parameters (height, weight and heart rate) has been proposed to improve the accuracy of CMR to noninvasively estimate pulmonary artery pressures¹³³, although there are concerns regarding the underlying mathematical methodology¹³⁴. A different mathematical model combining CMR-derived RV ejection fraction and pulmonary artery average velocity has been used as a non-invasive method for estimating PVR, with good correlation observed between invasively quantified and CMR-estimated PVR ($r = 0.84$, $p < 0.001$)¹³⁵. In their cohort of unselected treatment-naïve patients with suspected PH, Swift *et al* combined CMR-derived mPAP ($mPAP = -4.6 + (\text{interventricular septal angle} \times 0.23) + (\text{ventricular mass index} \times 163)$), with CMR-predicted PAWP from left atrial volumetry and CMR phase-contrast cardiac output, to non-invasively calculate PVR¹³⁶. Noninvasive CMR- and RHC-derived PVR correlated well ($r^2 = 0.76$), with CMR-estimated PVR identifying invasive PVR ≥ 3 Wood units (WU) with a high degree of accuracy (area under curve 0.94, $p < 0.0001$).

1.7 Treatment of Pulmonary Arterial Hypertension

1.7.1 General Measures and Supportive Therapy

The following general measures are commonly used in the treatment of pulmonary arterial hypertension:

- **Exercise** – A small study in patients with severe but stable symptomatic pulmonary hypertension has demonstrated that exercise and respiratory training could improve 6MWD and CPET performance, quality of life and NYHA functional class¹³⁷. There are a number of ongoing trials investigating the use of exercise training in patients with PAH¹³⁸, and current guidelines recommend that supervised exercise training should be considered in physically deconditioned PAH patients¹³⁹.
- **Oxygen** - Acute oxygen therapy can improve pulmonary haemodynamics in hypoxic and normoxic patients¹⁴⁰. Patients with resting arterial PaO₂ < 8kPa may be prescribed oxygen for at least 15 hrs per day. In-flight oxygen is recommended for pulmonary hypertension patients in functional class III and IV¹⁴¹, although the utility of hypoxic challenge testing in precapillary pulmonary hypertension remains unclear¹⁴².
- **Anticoagulation** - PAH is associated with abnormalities in coagulation and fibrinolytic pathways, and impaired platelet function. Anticoagulation may prevent vascular thrombotic lesions and pulmonary embolism^{106, 143}. Oral anticoagulant treatment should be considered in patients with IPAH, heritable PAH, and PAH due to use of anorexigens, and may be considered in associated PAH.
- **Diuretics** - In PAH there is excessive afterload, resulting in right ventricular dilatation and right heart failure. Diuretic therapy, often at high dosages, may benefit patients with significant fluid overload.
- **Arrhythmia Management** - Tachyarrhythmias are poorly tolerated, and often manifest with worsening dyspnoea, syncope or right heart failure^{56, 144}. Digoxin has been shown to improve cardiac output acutely in IPAH and may be given to slow ventricular rate in patients who develop atrial tachyarrhythmias.

- **Pregnancy** – Despite recent therapeutic advances, pregnancies in patients with PAH are associated with serious complications and high mortality rates. Consequently, all current guidelines strongly discourage pregnancy and recommend an effective method of contraception in women of childbearing age.²

1.7.2 Disease-Targeted Therapies for Pulmonary Arterial Hypertension

Acute vasoreactivity testing performed during right heart catheterisation is used to identify patients who may benefit from long term therapy with calcium channel blockers (CCB). Patients with a positive vasodilator response at RHC (approximately 10% of patients with idiopathic PAH) can be commenced on nifedipine (if there is a relative bradycardia) or diltiazem (if there is a relative tachycardia). Relatively high daily CCB dosages are used in this setting, 120–240 mg for nifedipine and 240–720 mg for diltiazem. Only half of the positive acute responders are also positive long term responders to calcium channel blockers¹⁰¹. If there is an inadequate clinical response to, or poor tolerance of, CCB therapy then additional PAH therapy should be instituted.

Until the 1990s, therapeutic options for pulmonary arterial hypertension were limited to supportive measures (oxygen, diuretics, anticoagulation) and lung transplantation. However, in the last two decades there have been a number of different classes of disease-targeted therapies developed to treat PAH, targeting different pathways. Prostacyclin and its analogues target the prostacyclin pathway, endothelin receptor antagonists target the endothelin-1 pathway, and phosphodiesterase-5 inhibitors and soluble guanylate cyclase activators target the nitric oxide pathway.

In the United Kingdom and Ireland, there are currently nine approved PAH targeted drugs with different routes of administration (Table 1.4), although the approval of additional drugs is expected in the near future.

Table 1.4 Approved PAH therapies in United Kingdom in 2015

Treatment	Route of administration
Epoprostenol	Intravenous
Iloprost	Inhaled / Intravenous
Treprostinil	Subcutaneous
Bosentan	Oral
Ambrisentan	Oral
Macitentan	Oral
Sildenafil	Oral
Tadalafil	Oral
Riociguat	Oral

1.7.2.1 Prostanoids

Prostacyclin is an arachidonic acid derivative, produced predominantly by endothelial cells and acting via the cyclic adenosine monophosphate pathway, which induces potent vasodilatation of the pulmonary vascular beds as well as having antithrombotic and antiproliferative effects¹⁴⁵. Patients with PAH have been shown to have abnormalities in prostacyclin metabolic pathways, with reduced prostacyclin synthase expression in the pulmonary arteries¹⁴⁶.

Epoprostenol is a synthetic prostacyclin available as a stable freeze-dried preparation for intravenous infusion. It must be dissolved in an alkaline buffer, has a very short half-life (3–5 min) and is stable at room temperature for only 8 h. Therefore, it needs to be administered continuously by means of an infusion pump via a tunnelled, cuffed central venous catheter. Treatment with epoprostenol is initiated at a dose of 2–4 ng/kg/min, with the dose increasing at a rate limited by side effects (flushing, headache, diarrhoea, jaw ache, hypotension). The optimal dose varies between individual patients, usually ranging between 20 and 40 ng/kg/min^{53, 147}. Serious adverse events related to the epoprostenol therapy include pump malfunction, local site infection, catheter obstruction, and sepsis. Abrupt interruption of the infusion should be avoided as it can lead to a rebound PH with symptomatic deterioration and death.

More stable prostacyclin analogues such as treprostinil and iloprost with similar pharmacodynamic effects are now available for the treatment of PAH. Treprostinil is a tricyclic benzidine analogue of prostacyclin, which is chemically stable and has a longer half-life than Epoprostenol. In the UK it is administered via a continuous subcutaneous infusion (although has been used in intravenous, inhaled and oral forms in the USA & Canada). The most common adverse effect of subcutaneous treprostinil is pain at the infusion site, leading to discontinuation of therapy or dose limitation dose¹⁴⁸. Treatment with subcutaneous treprostinil is initiated at a dose of 1–2 ng/kg/min, with doses increasing at a rate limited by side effects (site pain, flushing, headache) to an optimal dose usually between 20 and 80 ng/kg/min. Iloprost is a stable, synthetic analogue of prostacyclin that can be administered by the aerosolised route, so is theoretically selective for the pulmonary circulation. Inhaled Iloprost is administered by daily repetitive inhalations (6–9 times per day, 2.5 – 5 mg per inhalation) and is usually well tolerated, with flushing and jaw pain being the most frequent side effects.

1.7.2.2 Endothelin Receptor Antagonists

The endothelin system plays a prominent role in the pathogenesis of PAH, with activation of the endothelin system demonstrated in both the plasma and lung tissue of PAH patients^{149, 150}. Endothelin-1 is a potent vasoconstrictor that binds to two distinct receptor isoforms in the pulmonary vascular smooth muscle cells, endothelin-A and endothelin-B receptors, to regulate cell proliferation and vascular tone. The activation of endothelin-B receptors, present in endothelial cells, leads to the release of nitric oxide and prostacyclin that may counterbalance the deleterious effects of endothelin-1.

Bosentan is an oral active dual endothelin-A and endothelin-B receptor antagonist and was the first orally active agent to be granted regulatory approval in the treatment of PAH¹⁵¹. It is started at a dose of 62.5 mg twice daily, and is uptitrated to 125 mg twice daily after 4 weeks. Liver function tests should be performed monthly in patients receiving Bosentan as up to 10% of patients experience elevated hepatic transaminases, however these are usually reversible after dose reduction or discontinuation².

Ambrisentan is a selective endothelin-A receptor antagonist that has been approved for patients in WHO class II and III. It is commenced at 5mg once daily, increasing to 10mg once daily if the initial dosage is well tolerated. Ambrisentan is associated with a lower incidence of abnormal liver function tests than Bosentan, and has been used safely in a small group of patients who had previously discontinued Bosentan (or Sitaxentan) for this reason¹⁵². Monthly liver function testing is still required. An increased incidence of peripheral oedema has been noted with Ambrisentan.

Macitentan is a dual endothelin A/B receptor antagonist, chemically related to Bosentan, which has been recently received approval for use in WHO class II and III PAH in the UK. It has been shown to improve morbidity and mortality in a 24-month event-driven study of patients with PAH¹⁵³.

Sitaxentan, an oral endothelin-A receptor antagonist, was withdrawn from use in 2010 due to an association with fatal acute hepatic failure¹⁵⁴.

1.7.2.3 Phosphodiesterase type-5 Inhibitors and Soluble Guanylate Cyclase Activators

The pulmonary vasculature contains substantial amounts of phosphodiesterase type-5 (PDE-5), a cyclic guanosine monophosphate (cGMP) degrading enzyme. Endothelium dependent vasodilation is mediated by nitric oxide synthase in the pulmonary circulation, with endothelium derived nitric oxide (NO) diffusing into pulmonary smooth muscle cells, where it stimulates soluble guanylate cyclase to produce cGMP. Cyclic GMP promotes vascular smooth muscle relaxation via protein kinase G. PDE-5 inhibitors have been shown to augment nitric oxide-mediated vasodilatation, and also exert antiproliferative effects¹⁵⁵⁻¹⁵⁷.

Sildenafil is an orally active, potent and commonly used PDE-5 inhibitor that is effective in idiopathic PAH and PH associated with a range of conditions¹⁵⁸⁻¹⁶⁰. It is usually commenced at a dose of 20mg tid, but up-titration to 50-80mg tid is frequently performed. The most common side effects are headaches, flushing and epistaxis, although it can rarely be associated with sensorineural hearing loss¹⁶¹.

Tadalafil is a less commonly used once daily PDE-5 inhibitor, with a similar side-effect profile to Sildenafil.

Riociguat is the first in a new class of agents known as soluble guanylate cyclase (sGC) activators. It has a dual mode of action, increasing the sensitivity of soluble guanylate cyclase to nitric oxide, and directly stimulating soluble guanylate cyclase independently of nitric oxide¹⁶². Riociguat has been shown to improve exercise capacity in PAH, and to improve exercise capacity and pulmonary vascular resistance in inoperable or persistent (post-endarterectomy) CTEPH^{163, 164}.

1.7.2.4 Disease-targeted Therapy in Connective-Tissue Disease Associated Pulmonary Arterial Hypertension

Despite similar baseline clinical and haemodynamic characteristics, outcomes in CTD-PAH are worse than in IPAH¹⁶⁵. PAH therapies have been widely studied in patients with connective tissue disease, although often as a subgroup analysis of clinical trials containing patients with PAH of all causes. Nifedipine was found to produce an acute and sustained reduction in pulmonary vascular resistance in a small group of patients with CTD-PAH¹⁶⁶. In a large study of 111 patients with scleroderma and moderate-to-severe pulmonary hypertension, twelve weeks of intravenous Epoprostenol was found to improve exercise capacity (placebo-corrected increase in 6MWD of +108m), haemodynamics and functional class, although there was no difference in survival¹⁶⁷. Similar symptomatic and haemodynamic improvements were observed with subcutaneous Treprostinil therapy, although improvements in 6MWD were more modest (placebo-corrected increase of +25m)¹⁶⁸. Patients with SSc-PAH treated with bosentan monotherapy were less likely to improve NYHA functional class, and had higher mortality rates than a similar group of bosentan-treated IPAH patients¹⁶⁹. In a post-hoc analysis of the SUPER-1 study, Sildenafil at a dose of 20mg tid was found to improve exercise capacity, functional class and pulmonary vascular resistance in 84 patients with CTD-PAH¹⁷⁰. The magnitude of treatment effects in CTD-PAH is often smaller than in IPAH, and this may be due to the difficulties associated with performing 6MWT in the CTD group. Patients with CTD may have coexisting interstitial lung disease, left heart disease or musculoskeletal problems, which could all limit any potential improvement in 6MWD.

The overall effect of PAH therapy in CTD-PAH has therefore been assessed by meta-analysis. In a review of 10 randomized controlled trials, containing patients with CTD-PAH treated with bosentan, sitaxentan or sildenafil, there was an absence of clinically relevant improvement on exercise capacity (as measured by 6MWD) in patients with CTD/SSc after 12 to 18 weeks of treatment¹⁷¹. When examining the impact of modern PAH therapy (oral therapies) on IPAH and SSc-PAH, Rubenfire *et al* found that survival rates in their centre of patients with SSc-PAH presenting in the “modern era” did not differ from pre-2002, when the sole PH-specific treatment was Epoprostenol. Patients with SSc-PAH had a lower probability of treatment with a prostacyclin, and were less likely to be taking warfarin, when compared with IPAH patients¹⁷². This finding was in keeping with data from the large US-based REVEAL cohort, where patients with CTD-PAH were less likely to be on prostacyclin (36.3% vs 46.9%, $p < 0.0001$) and combination PAH therapy

(39.5% vs 45.0%, $p = 0.03$) than those with IPAH¹⁷³. Despite these findings it is still generally felt that, in contrast to patients treated historically with basic drugs and prostanoids, patients treated in the present day had improved survival associated with a lack of deterioration in cardiac haemodynamic function^{174, 175}. This is supported by data from the 2015 UK National Pulmonary Hypertension Audit which demonstrated similar survival rates in IPAH and CTD-PAH in the current treatment era (<http://www.hscic.gov.uk/ph>).

The use of immunosuppressant therapy is more common in CTD-PAH than in IPAH, with highest rates of use in SLE, MCTD and RA rather than in systemic sclerosis¹⁷³. Combination therapy with glucocorticosteroids and cyclophosphamide may result in clinical improvement in patients with PAH associated with SLE or MCTD¹⁷⁶. In patients with advanced PAH (i.e. PVR ≥ 10 , already on ≥ 2 PAH therapies), the tyrosine kinase inhibitor Imatinib was found to improve exercise capacity and pulmonary haemodynamics, but resulted in serious adverse events and drug discontinuations¹⁷⁷.

1.7.2.5 Use of Pulmonary Arterial Hypertension Therapies in Left Heart Disease

The use of PAH-specific therapies in PH due to left heart disease has not been as successful as their use in IPAH. There are no long-term studies examining the use of prostanoids solely in PH-HFpEF, however in the Flolan International Randomized Trial (FIRST), long-term intravenous prostacyclin was associated with increased mortality in patients with advanced left ventricular failure with or without PH, despite improvements in cardiac output and PAWP¹⁷⁸.

Long-term use of the endothelin-receptor antagonist Bosentan in patients with PH-HFpEF was not associated with improvements in pulmonary haemodynamics, but was associated with an increased rate of serious adverse events¹⁷⁹. Some small studies have suggested that the phosphodiesterase-5 inhibitor Sildenafil may potentially improve pulmonary haemodynamics in patients with heart failure and elevated PAP, without detrimental systemic haemodynamic consequences¹⁸⁰. However, in the RELAX study of 216 patients with HFpEF (and median PASP of 41mmHg), twenty-four weeks of Sildenafil therapy did not cause a significant improvement in exercise capacity or clinical status¹⁸¹.

In a Phase IIb study, the soluble guanylate cyclase activator Riociguat was well tolerated in patients with PH caused by left ventricular systolic dysfunction and improved cardiac index and pulmonary and systemic vascular resistance, although did not significantly alter 6MWD¹⁸². In patients with PH-HFpEF, a single acute dose of Riociguat was well tolerated, had no significant effect on mPAP, and improved exploratory haemodynamic and echocardiographic parameters¹⁸³.

PAH-specific therapies are expensive (e.g. endothelin receptor antagonist therapy is approximately £20,000 per patient per annum), and potentially dangerous, so current guidelines do not recommend their use in PH-HFpEF.

1.8 Changing Demographics in Pulmonary Arterial Hypertension

There is growing recognition that the demographics of pulmonary arterial hypertension have changed from the traditional phenotype of the “young female” observed in early registries of primary pulmonary hypertension in the 1980s and 1990s. In the initial NIH (National Institutes of Health) registry published in 1987, the mean age of patients was 36 yrs, with a female-to-male ratio of 1.7:1⁵⁴.

In the French registry, 674 cases of PAH presenting at seventeen university pulmonary vascular centres between October 2002 and October 2003 were prospectively enrolled and followed up for three years⁸¹. The mean age of the whole PAH population was 50yrs (idiopathic PAH = 52 yrs), with a quarter of cases occurring over the age of 60, and some patients diagnosed in their 80s. In the USA-based REVEAL registry, the mean age of idiopathic PAH at diagnosis was 50 years, approximately 5% of the patients were diagnosed at ≥ 75 years of age, and many patients had significant comorbidities including systemic hypertension (42%), obesity (38%), sleep apnoea (27%), obstructive airway disease (23%), thyroid disease (20%), diabetes (14%) and ischaemic cardiovascular event (10%)¹⁸⁴.

The Pulmonary Hypertension Registry of the United Kingdom and Ireland was the first major national study of incident, treatment-naïve patients with IPAH, and consisted of 482 newly diagnosed IPAH patients from 2001 to 2009¹⁸⁵. The median age of the group was 50 years (IQR, 36–65) and 13.5% (n = 65) of patients were older than 70 years of age at the time of diagnosis. Patients diagnosed at > 50yrs of age had a higher incidence of ischaemic heart disease (24% v 1%), systemic hypertension (42% v 11%), atrial fibrillation (11% v 0%), diabetes (23% v 5%) and hypothyroidism (16% v 8%) than those diagnosed at ≤ 50 yrs. In a comparison of baseline characteristics according to year of diagnosis, patients were stratified into three cohorts based on the date of diagnostic RHC (2001-2003, 2004-2006, and 2007-2009)¹⁸⁵. Patients diagnosed in the latest cohort (2007-2009) were older (52 v 45 yrs), had higher BMI (29.8 v 28.1) and more comorbidities than those diagnosed in the earliest cohort (2001-2003).

These registry findings demonstrate that IPAH is being increasingly diagnosed in older patients, who often have coexisting cardiovascular disease or other comorbidities. Using data from the multi-centre COMPERA (Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension) registry, Hoepfer *et al* compared

demographics, clinical characteristics, haemodynamics, treatment patterns and outcomes of younger (18–65 years) and elderly (>65 years) patients with newly diagnosed IPAH¹⁸⁶. The median (IQR) age at diagnosis for the whole patient population was 71 (16) years, with a mean age of 65 years. Younger patients (n=209; median age, 54 [16] years) showed a female-to-male ratio of 2.3:1 whereas the gender ratio in elderly patients (n=378; median age, 75 [8] years) was only 1.2:1. At baseline RHC, there was no difference in right atrial pressure or cardiac index between the groups, however PAWP was higher (10 v 9 mmHg, $p < 0.001$) and mPAP (41 v 50 mmHg, $p < 0.001$) and PVR (8.3 v 12.0 Wood units) were lower in the older patient population. Compared with younger patients, elderly patients were less likely to receive combination PAH therapy, less likely to reach current treatment targets, and had lower survival rates.

Whilst it is clear that IPAH is now being diagnosed more frequently in older people, it remains a rare condition, and pulmonary hypertension in this age group is usually the result of underlying left heart or lung disease. Pulmonary artery systolic pressure measured by echocardiography increases with age, with increasing PASP associated with higher mortality in patients with and without cardiopulmonary disease¹⁸⁷.

Pugh *et al* investigated the underlying cause of pulmonary hypertension in 246 elderly (>65yr) patients who had been referred for diagnostic assessment between 1995 and 2011, of which 202 had PH¹⁸⁸. PAH (WHO group 1) was diagnosed in 36 patients (15%), of which only 4 patients had idiopathic PAH. PH owing to left-sided heart disease (WHO group 2) was the most common type of PH in the cohort (70 patients, 28%), most of these patients had no significant valvular heart disease and a left ventricular ejection fraction of greater than 50%, and therefore diagnosed as PH-HFpEF. Comorbid conditions were common throughout the study population, with cardiovascular and metabolic diseases seen most commonly in patients with WHO group 2 PH. It is worth noting that the “WHO group 2 PH” cohort in this study contained patients with an initial normal PAWP, but who had an increase in PAWP > 7 mmHg to > 15 mmHg after IV fluid challenge. By contrast, patients with evidence for Group 2 disease with PH that was believed to be “out of proportion” (i.e. transpulmonary gradient ≥ 15 mm Hg) were classified as “other/mixed PH”.

1.9 Left Ventricular Diastolic Dysfunction and Heart Failure with Preserved Ejection Fraction

Left ventricular diastolic dysfunction is common within the general population, with community-based echocardiography studies reporting that in patients aged over 45yrs 20.8% had mild diastolic dysfunction, 6.6% had moderate diastolic dysfunction and 0.7% had severe diastolic dysfunction¹⁸⁹. The prevalence of diastolic dysfunction increased with age, was equally common in men and women, and was more common in patients with known cardiovascular disease (including LV systolic dysfunction) or diabetes. LV systolic dysfunction was less common than diastolic dysfunction, with only 6.0% having an LV ejection fraction of $\leq 50\%$ and 2.0% $\leq 40\%$, and 5.6% of the study population had moderate or severe diastolic dysfunction with a normal ejection fraction. Diastolic dysfunction was predictive of all-cause mortality even after controlling for age, sex and LV ejection fraction.

Heart failure with preserved ejection fraction (HFpEF) describes the entity of clinical heart failure in the setting of a left ventricular ejection fraction of more than 50 percent, and is felt to be primarily the result of left ventricular diastolic dysfunction^{190, 191}. HFpEF is currently observed in approximately 50% of heart failure patients, with a prevalence rising by 1% per year compared with heart failure due to reduced ejection fraction (HFrEF)^{192, 193}. Patients with HFpEF are more likely to be older and female and to have a history of hypertension and atrial fibrillation than those with HFrEF¹⁹⁴. Outcomes in HFpEF are similar to that seen in HFrEF (1year mortality: HFpEF 22% v HFrEF 26 %), even after adjusting for age, sex and comorbidities.

1.9.1 Pulmonary Hypertension in Heart Failure with Preserved Ejection Fraction

Pulmonary hypertension is frequently associated with left heart disease, with increased left-sided filling pressure causing a ‘passive’ increase in pulmonary artery pressure (PAP), which can eventually lead to pulmonary vasoconstriction and remodelling of the small-resistance pulmonary arteries¹⁹⁵. The most recent World Health Organization classification identified four different categories: PH secondary to left ventricular systolic dysfunction, PH resulting from left ventricular diastolic dysfunction, PH due to left-sided valvular disease, and PH associated with congenital/acquired left heart inflow/outflow obstruction and congenital cardiomyopathies⁵. The most common type of left heart disease that causes PH is HFpEF (PH-HFpEF).

PH-HFpEF is usually defined as mean PAP > 25 mm Hg in the presence of pulmonary artery wedge pressure (PAWP) > 15 mm Hg, signs and symptoms of heart failure, left ventricular ejection fraction > 50%, and absence of significant left-sided valvular heart disease¹⁹⁶. It has been proposed that PH due to left heart disease, including PH-HFpEF, can be further categorized depending on the presence or absence of a “precapillary” or intrinsic pulmonary vascular component. In isolated post-capillary PH-HFpEF the transpulmonary gradient (TPG) and PVR are usually within normal limits (TPG < 12 - 15 mmHg and PVR < 2.5 - 3 Wood units). In combined post- and precapillary PH or “mixed PH” the superimposed precapillary component increases the PAP disproportionate to the left-sided filling pressure, resulting in an increased TPG or PVR (TPG ≥ 12 - 15 mm Hg and PVR ≥ 2.5 - 3 Wood units)¹⁹⁷. However, there are concerns that TPG and PVR do not truly represent intrinsic pulmonary arteriolar remodelling, and that the diastolic pulmonary gradient (DPG: diastolic PAP - PAWP) should be used instead¹⁹⁸. A new proposed categorisation based on DPG would therefore be: isolated postcapillary (mean PAP > 25 mm Hg, PAWP > 15mm Hg, and DPG < 7 mm Hg) and combined postcapillary and precapillary PH (mean PAP > 25 mm Hg, PAWP > 15 mm Hg, and DPG ≥ 7 mmHg)¹⁹⁹.

1.9.2 Prevalence of Pulmonary Hypertension in Heart Failure with Preserved Ejection Fraction

The prevalence of pulmonary hypertension among the overall HFpEF population varies depending on the haemodynamic threshold used to define PH, the diagnostic modality (echocardiography or RHC) used, and the patient group studied. In a retrospective analysis of patients hospitalized with HFpEF between 1999 and 2001, “moderate” pulmonary hypertension at echocardiography (mean RVSP of 47 ± 17 mm Hg) was observed in 272 of the 619 patients²⁰⁰. In their retrospective review of cardiac catheterisation data from 455 patients with HFpEF from 1996 to 2007, Leung *et al* found that 239 patients (52.5%) had PH as defined by an mPAP of >25 mmHg²⁰¹. In a study investigating baseline haemodynamics and response to vasodilator therapy in HFpEF and HFrEF, both groups had elevated mPAP (41 vs 40 mm Hg, $p = 0.50$) and PASP was higher in HFpEF than HFrEF (64 vs 59 mm Hg, $p = 0.02$)²⁰².

In a community-based study, Lam *et al* found that 83% of patients with HFpEF had pulmonary hypertension compared with only 8% of a control group of hypertensive individuals without heart failure, and that the PASP strongly predicted mortality in HFpEF²⁰³. However, in this study the cutoff to define PH was low (a PASP of >35 mmHg at echocardiography), especially given that normal PASP increases with age²⁰⁴. Even after adjusting for PAWP, PASP was still higher in the HFpEF group than in the hypertensive controls, suggesting that a pre-capillary component was contributing to greater PH in HFpEF. In 548 subjects with HFpEF and a variable degree of PH and RV dysfunction, followed for up to ten years, a worse prognosis was observed for patients with PASP above the median (>47 mm Hg) and RV systolic dysfunction²⁰⁵. RV systolic dysfunction was a significant predictor of mortality even after adjustment for age and PASP.

In a clinical trial setting, only 36% of patients with HFpEF (left ventricular ejection fraction $\geq 45\%$) enrolled in the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) study had a baseline tricuspid regurgitant jet velocity > 2.9 m/s (equivalent to a PASP > 35 mmHg) at echocardiography²⁰⁶. TR jet velocity was significantly related to the E/E' ratio, and an elevated TR velocity in the absence of elevated E/E' was uncommon, occurring on only 5% of participants. After a median follow-up of 2.9 years, a higher pulmonary artery pressure was predictive of the composite outcome heart failure hospitalization, cardiovascular death, or aborted cardiac arrest²⁰⁷. In the RELAX trial, investigating the effect of Sildenafil in 216 stable outpatients

with HFpEF (ejection fraction $\geq 50\%$), the median PASP for the entire study population at baseline was 41mmHg¹⁸¹.

1.9.3 Pathophysiology and Pathogenesis of Pulmonary Hypertension in Heart Failure with Preserved Ejection Fraction

HFpEF is a heterogeneous syndrome, with a range of phenotypes and pathophysiologies, which result in increased left ventricular filling pressures and the clinical picture of heart failure²⁰⁸. In HFpEF there is normal systolic LV function but impaired diastolic LV function, consisting of prolonged relaxation, slow filling and increased diastolic stiffness^{191, 209}. Invasive studies have demonstrated abnormalities in active relaxation and passive stiffness of the LV in patients with HFpEF²¹⁰.

LV diastolic dysfunction usually results from increased myocardial stiffness, either within the extracellular matrix or the cardiomyocytes. Stiffness of the extracellular matrix can be the result of a number of collagen abnormalities, including excessive synthesis and impaired degradation of collagen type 1²¹¹, and decreased matrix degradation due to downregulation of matrix metalloproteinases (MMPs) and upregulation of tissue inhibitors of matrix metalloproteinases (TIMPs)²¹². Intrinsic cardiomyocyte stiffness also contributes to diastolic LV dysfunction in HFpEF^{213, 214}. Abnormal slowing of LV relaxation, which can result in a reduction in LV stroke volume particularly at high heart rates, is also prominent in HFpEF and may be associated with a myocardial energy deficit²¹⁵. It is also recognised that there are other contributors to disease pathophysiology in HFpEF, including potential LV myocardial contractile dysfunction²¹⁶, impaired ventricular–vascular coupling^{217, 218}, chronotropic incompetence²¹⁹ and abnormal exercise-induced and flow mediated vasodilation¹⁹¹.

Increased LV filling pressures, irrespective of LV systolic function, lead to pulmonary venous hypertension and postcapillary PH²²⁰. The histological changes that are observed in postcapillary PH include enlarged and thickened pulmonary veins, pulmonary capillary dilatation, interstitial oedema, alveolar haemorrhage, and lymphatic vessel and lymph node enlargement²²¹. Additional abnormalities of the pulmonary vasculature observed in “mixed / pre-capillary and post-capillary” PH-HFpEF include thickening of the alveolar-capillary membrane and remodelling of the small pulmonary arteries, with luminal occlusion, medial hypertrophy, and intimal and adventitial fibrosis¹⁹⁵. The angioproliferative plexiform lesions typical of PAH are rare in PH-HFpEF. In pathological specimens from patients with PH due to left heart disease, Gerges *et al* demonstrated a direct correlation between TPG / DPG and the degree of smooth muscle proliferation in resistance pulmonary arteries, and that patients with combined mixed PH-HFpEF with a

DPG > 7 mmHg had an increased proportion of small pulmonary arteries with medial hypertrophy, intimal fibrosis, adventitial fibrosis, and luminal occlusion compared with those with a DPG of < 7 mmHg²²².

Most patients with HFpEF who develop PH will have isolated postcapillary PH, with only a small minority developing combined precapillary and postcapillary PH. Proposed risk factors for developing the “precapillary” pulmonary arterial vasoconstriction and remodelling include increasing age²⁰³ and female gender^{223, 224}, whilst atrial arrhythmias, obesity and chronic obstructive pulmonary disease are associated with an overall increased risk for developing PH in HFpEF²⁰¹.

As pulmonary artery pressure increases in HFpEF there is a progressive increase in RV afterload, which can eventually result in RV failure. Right ventricular dysfunction is less common in HFpEF than in HFrEF²²⁵, however estimates of prevalence vary widely (from as low as 4%²⁰⁶ to as high as 33%²²⁶ of patients with HFpEF). Increased pulmonary artery pressure and RV dysfunction are associated with increased mortality in HFpEF. Kjaergaard *et al* demonstrated that patients with HFpEF and a PASP at echocardiography of > 39 mmHg had an increased mortality compared with those with PASP of < 39 mmHg²²⁷. In HFpEF, RV dysfunction is associated with an additional increased risk of adverse outcomes beyond the risk conferred by PH alone, with an RV Fractional Area Change of < 35% equating to a 2.4-fold increase in mortality compared with patients without RV dysfunction, independent of systolic PAP²²⁶. Similarly, increasing RV hypertrophy is also associated with higher mortality in HFpEF²²⁸.

1.10 Hypotheses and Aims of Thesis

The overall aim of this thesis was to determine if cardiac magnetic resonance imaging could be used to help address three important contemporary issues in the diagnosis and determination of outcome in pulmonary hypertension.

These are:

- (1) Is there a non-invasive way to distinguish idiopathic pulmonary arterial hypertension (rare) from pulmonary hypertension due to heart failure with preserved ejection fraction (common)?
- (2) Do disease-targeted PAH therapies improve right ventricular function?
- (3) Does impaired cardiac function explain why survival is worse in connective-tissue disease associated PAH than in idiopathic PAH?

To answer these questions, three separate, but related studies were performed; see Chapters 3, 4 and 5. The hypothesis of each study is explained below.

Chapter 3. ‘Left atrial volume by CMR distinguishes IPAH from PH-HFpEF’. HFpEF due to left ventricular diastolic dysfunction is common within the general population, and is often associated with PH^{192, 193, 203}. Meanwhile, the average age at diagnosis of IPAH has increased over recent decades, with registry data now suggesting a mean age of 50 years^{81, 185}. Distinguishing between IPAH and PH-HFpEF can therefore be difficult on clinical grounds⁵⁷, and invasive RHC is currently performed by PH centres to achieve this². Left atrial enlargement, a marker of LV diastolic dysfunction, is commonly observed in PH-HFpEF but less so in PAH^{57, 229}. The principal hypothesis of this study was that left atrial volume measured using CMR imaging would be able to distinguish IPAH from PH-HFpEF.

Chapter 4. ‘Changes in RV function measured by CMR imaging in patients receiving PAH-targeted therapy’. Right ventricular function is an important predictor of survival in PAH^{1, 230, 231}. The last 2 decades have seen the introduction of several treatments targeting

the pathophysiological mechanisms of PAH, however their approval is largely based on improved haemodynamic variables at RHC, or improved exercise capacity measured with 6MWT^{2, 232}. The principal hypothesis of this study was that direct assessment of RV function using CMR would be an appropriate way of determining response to therapy and monitoring disease progression in PAH.

Chapter 5. 'Prognostic value of cardiac dysfunction in IPAH and CTD-PAH'. Despite similar baseline clinical and haemodynamic characteristics, outcomes in CTD-PAH are worse than in IPAH, with increased mortality and poorer response to disease-targeted PAH therapy^{165, 171, 233, 234}. The principal hypothesis of this study was that right and left sided cardiac dysfunction could influence prognosis and explain the survival difference between these two conditions.

Chapter 2

Materials and Methods

2.1 Patient Recruitment

The patients involved in the studies in this thesis were recruited during their diagnostic assessment for suspected pulmonary hypertension at the Western Infirmary, Glasgow UK (pre-August 2008) or the Golden Jubilee National Hospital, Clydebank, UK (post-August 2008). The clinical studies conducted in this thesis were reviewed and approved by the West Glasgow Research Ethics Committee. The patient consent form and information sheet are shown in Appendix 1. Patients were also recruited into the EURO-MR study from PH centres in: Amsterdam, Netherlands; Graz, Austria; Rome, Italy.

All patients had either been assessed first in the out-patient pulmonary vascular clinic and offered diagnostic admission for further assessment, or admitted directly for assessment following faxed or telephone referral from other hospitals within Scotland. All referrals were vetted by a Consultant from the Scottish Pulmonary Vascular Unit (Professor Andrew Peacock, Dr Martin Johnson),

2.2 Routine Diagnostic Assessment

2.2.1 Non-invasive Assessment

During the diagnostic admission, all patients underwent a series of investigations as part of the recommended diagnostic algorithm for PH². These included routine blood tests, arterial blood gas analysis, 12-lead electrocardiogram, chest radiograph, transthoracic echocardiogram, lung function and six-minute walk tests, ventilation/perfusion (V/Q) scan, High Resolution CT scan and a CT Pulmonary Angiogram (CTPA). The CTPA was not performed if the examination had been performed recently at the referring hospital, or if the patient had significant renal dysfunction. These investigations were performed at either the Western Infirmary and Gartnavel General Hospital, or the Golden Jubilee National Hospital.

The 6-minute walk test was performed in accordance with current guidelines⁷⁶.

Assessment of functional class was made in accordance with WHO guidelines (Appendix 2)

2.2.2 Right heart catheterisation

Right heart catheterisation was performed on the Thursday of the diagnostic admission in the cardiac catheterisation lab of the Golden Jubilee National Hospital (or Western Infirmary if pre-August 2008). For patients on regular diuretics or calcium channel blockers, these were stopped the day before. An 8F introducer sheath was inserted in the right internal jugular vein under ultrasound guidance, either on the Wednesday afternoon, or at the time of cardiac catheterisation. Right heart catheterisation was performed using a 7F triple-channel thermodilution Swan Ganz catheter (Baxter Healthcare, Irvine, California, USA). Pre-medication or sedation was not routinely used. The external pressure transducer was zeroed at the mid-thoracic line with the patient in the supine position, halfway between the anterior sternum and the bed surface, representing the level of the left atrium. Measurements were recorded with the patient in a supine position, at rest, breathing room air (unless supplemental oxygen was already in use prior to the procedure). All pressure measurements were determined at the end of normal expiration. These included mean right atrial pressure (RAP), right ventricular pressure and systolic and diastolic pulmonary artery pressures (PAP). Mean PAP was determined as the area

under the PAP trace. Pulmonary artery wedge pressure (PAWP) was recorded with the catheter in the wedge position and the balloon inflated. Cardiac output (CO) was determined in triplicate by the thermodilution technique, allowing the determination of pulmonary vascular resistance (PVR) by the following: $(\text{mean PAP} - \text{PAWP})/\text{CO}$. Cardiac Index was determined as $\text{CO} / \text{body surface area}$.

If the right heart catheterisation suggested pulmonary arterial hypertension, then pulmonary vasoreactivity testing was performed. Patients inhaled a mixture of nitric oxide (dose 10-20 parts per million) and oxygen for 5 minutes, and pulmonary haemodynamic measurements were repeated. A positive acute response was defined as a reduction of mean PAP ≥ 10 mmHg to reach an absolute value of mean PAP < 40 mmHg with an increased or unchanged CO.

The V/Q scan and CTPA had been reviewed by our specialist thoracic radiologist (Dr Mike Sproule, Gartnavel General Hospital) prior to right heart catheterisation, and if there was suspicion of chronic thromboembolic disease then pulmonary angiography was also performed.

2.2.3 Cardiovascular Magnetic Resonance Imaging

The CMR scans in this thesis were performed at the Western Infirmary or the Golden Jubilee National Hospital. Before August 2008 the scans were performed at the Western Infirmary by Ms Tracy Steedman, Cardiac MR Radiographer, and one of the previous SPVU clinical research fellows (Dr Kevin Blyth, Dr Lindsey McLure). After August 2008 scans were performed at the Golden Jubilee National Hospital by Ms Rosemary Woodward, Mr Andrew Saul or Mrs Carolyn Clark, Cardiac MR Radiographers. At both sites, scans were performed on a 1.5 T Siemens Sonata whole body MR scanner.

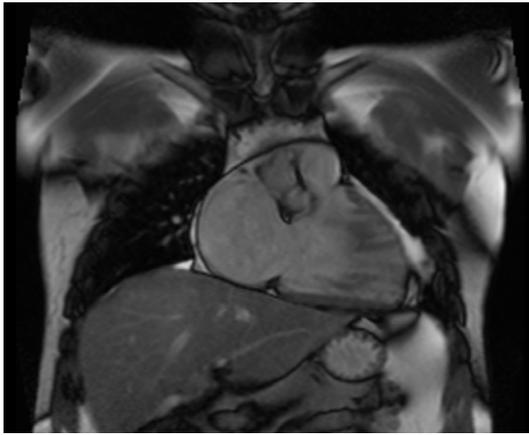
2.2.3.1 Patient preparation and positioning

Prior to entering the CMR scanning room, the patient was asked to complete and sign a safety questionnaire to ensure there was no contraindication to performing CMR. After changing into a hospital gown, the patient was asked to lie supine on the CMR examination table, which had been remotely removed from inside the magnet bore. Three-lead ECG monitoring pads were connected, and the phased array chest coil placed on the patient's chest and secured with a Velcro strap. Protective ear-defenders were fitted and an emergency buzzer supplied. The centre of the chest coil, approximating the position of the heart, was defined by a laser pointer attached to the magnet's inner circumference at the 12 o'clock position. This reference point was obtained to allow the patient to be moved within the bore of the magnet, to the point where their heart was at the centre of the main magnetic field. Once the patient was inside the bore of the magnet, the scanning room door was sealed and the staff performing the scan returned to the control room. Microphone and headphone function, allowing direct communication between patient and the CMR operator, was verified before image acquisition commenced.

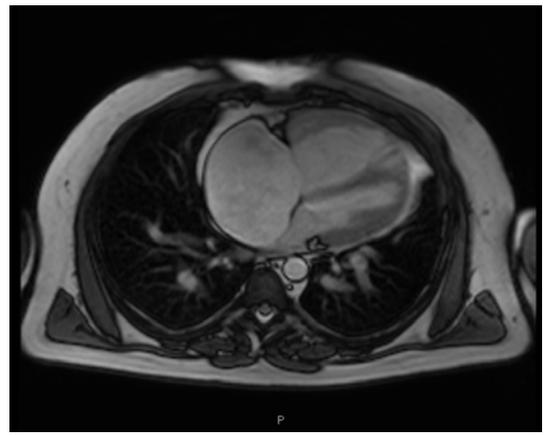
2.2.3.2 CMR Image acquisition

A standard CMR core imaging protocol was followed in all subjects. Steady state procession sequences (True FISP) were used to generate the initial axial scout images in the coronal, transverse and sagittal planes, allowing the heart to be localised within the thoracic cavity, and were used for planning subsequent cine images (Figure 2.1).

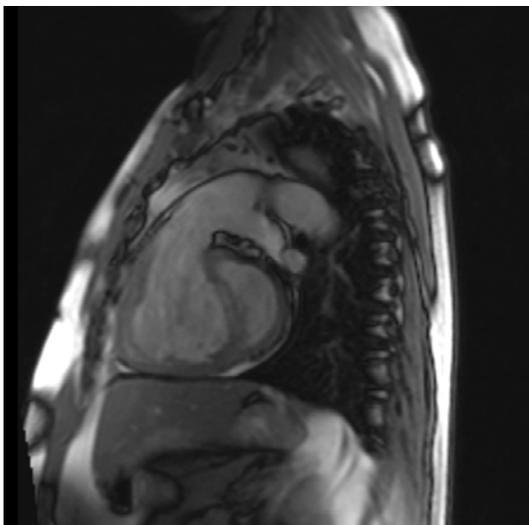
Figure 2.1 CMR scout images in the coronal, transverse and sagittal planes



Coronal Scout Image



Transverse Scout Image



Sagittal Scout Image

Typical axial scout images used to localise the heart within the thoracic cavity during CMR scanning of a patient with PH. Note the low definition images of these preliminary fast imaging with steady state procession (TrueFISP) scout images.

Vertical- and horizontal-long axis (VLA and HLA) cines (Figures 2.2 and 2.3) were planned and acquired based on the initial scout images.

Figure 2.2 Image 1 of a vertical long axis cine

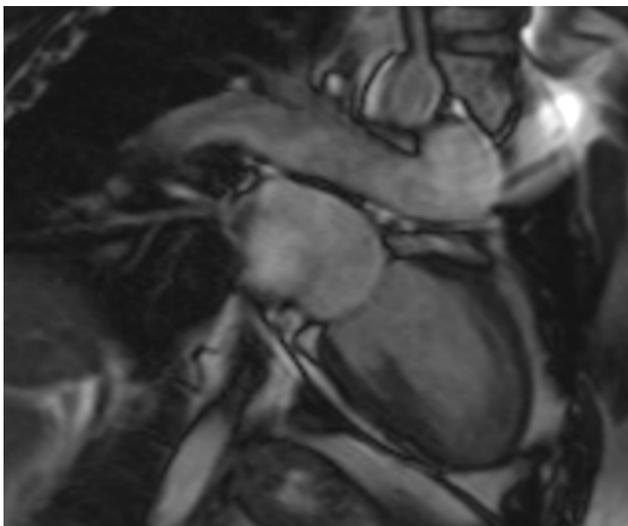


Image 1 of a vertical long axis fast imaging with steady state precession (TrueFISP) cine acquired in a patient with PH.

Figure 2.3 Image 1 of a horizontal long axis cine

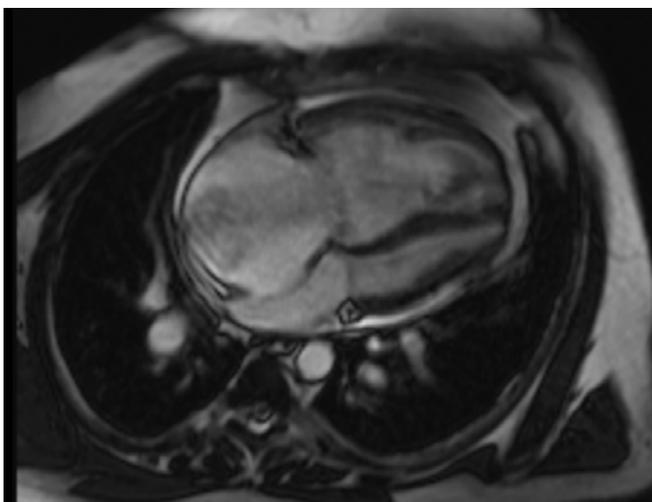
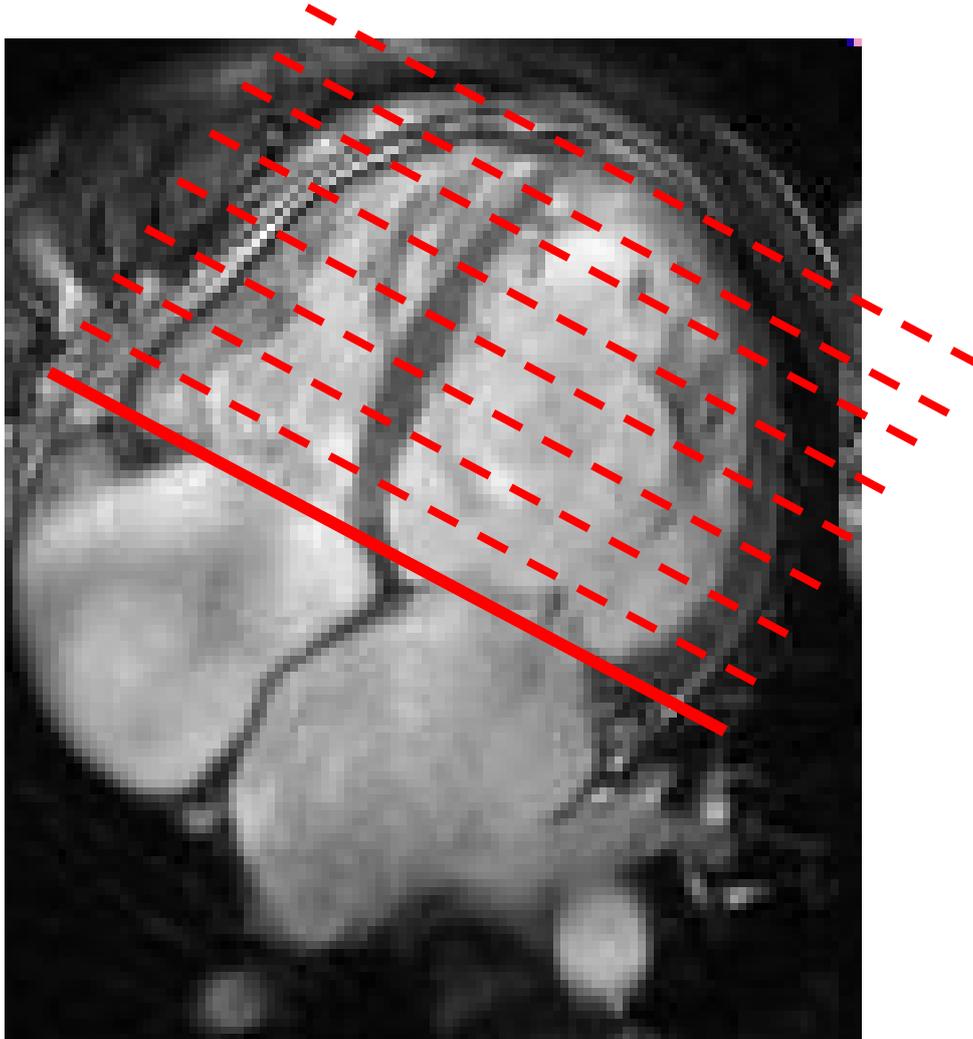


Image 1 of a horizontal long axis fast imaging with steady state precession (TrueFISP) cine acquired in a patient with IPAH. Note the right ventricular and right atrial enlargement, and the right ventricular hypertrophy.

A series of short axis (SA) cines was then planned on image one of the HLA cine, intersecting the atrioventricular valve roots on this view. The SA imaging plane was then propagated towards the cardiac apex, covering both ventricles with 8-mm SA imaging slices, separated by a 2-mm inter-slice gap (Figure 2.4).

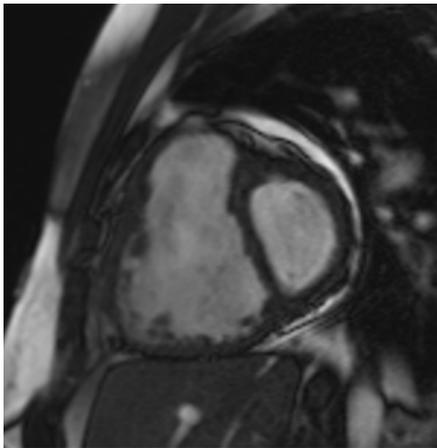
Figure 2.4 Horizontal long axis cine with planned short axis imaging slices



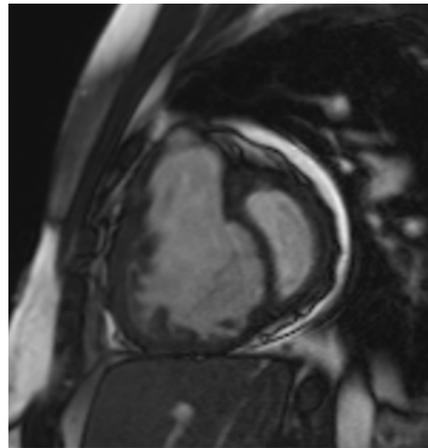
A horizontal long axis fast imaging with steady state precession (TrueFISP) cine acquired in a patient with PH due to left heart disease. This image was used to plan the first of a series of short axis (SA) cines, intersecting the atrioventricular valve roots. The SA imaging plane was then propagated apically, covering both ventricles with 8-mm SA imaging slices (red lines), separated by a 2-mm inter-slice gap.

A traditional left ventricle (LV) short axis cine stack was used for the acquisition of both RV and LV images (Figure 2.5). This stack of short axis images was used for the measurement of ventricular volume and mass using manual planimetry, at end-diastole and end-systole.

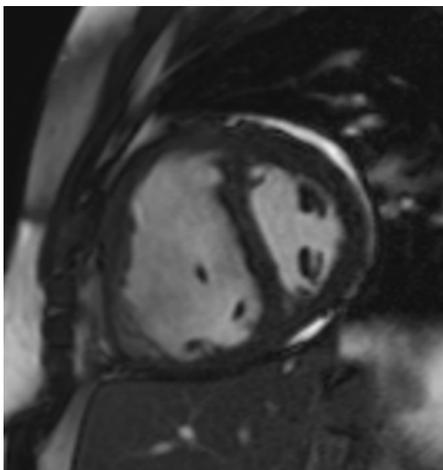
Figure 2.5 Basal, middle and apical short-axis cine images at end-systole and end-diastole



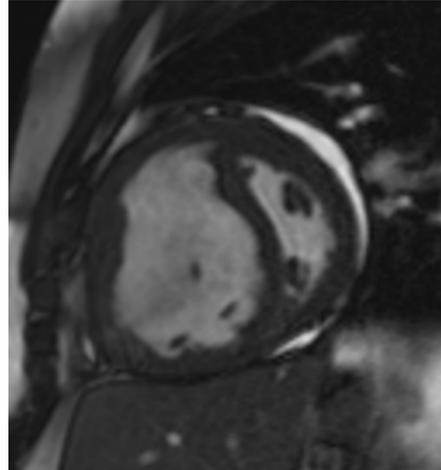
Basal (End-Diastole)



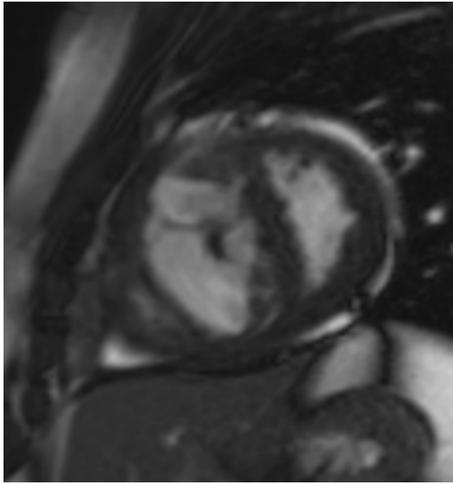
Basal (End Systole)



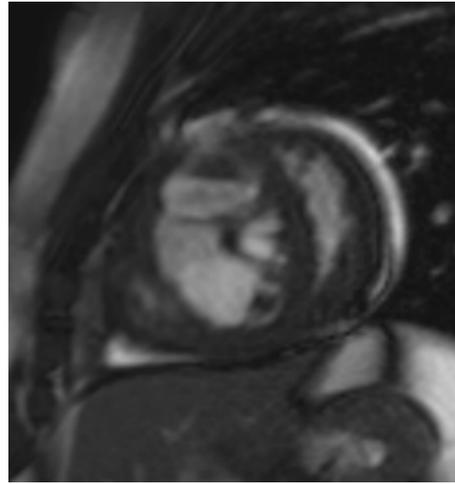
Middle (End-Diastole)



Middle (End Systole)



Apical (End-Diastole)



Apical (End Systole)

End-diastolic and end-systolic images from short axis cines acquired in a patient with IPAH. A stack of these images were acquired covering both ventricles from base to apex. Note the right ventricular dilatation, right ventricular hypertrophy, bowing of the interventricular septum at end-systole, and the presence of a pericardial effusion

Imaging parameters, which were standardised for all subjects, included: TR/TE/flip angle/voxel size/FoV = 3.14ms/1.6 ms/60°/2.2 x 1.3 x 8.0 mm/340 mm. Whenever possible, retrospective ECG-gating was used. This involves continuous data collection and the assignment of the collected data to appropriate points in the cardiac cycle retrospectively. Although this means that a mean cardiac cycle must be interpolated, because of natural variation in R-R interval, it ensures complete coverage of the cardiac cycle.

2.2.3.3 Common problems encountered during CMR imaging

The most common problem was that some patients could not hold their breath long enough to accommodate a complete pulse sequence. This was most commonly observed in patients who were already oxygen-dependent at the time of diagnostic assessment. In extreme cases imaging was performed with the patient breathing freely. However, in most the TR of the sequences could be shortened by altering the flip angle and/or reducing the phase oversampling used as standard in the imaging sequences.

Other problems encountered include claustrophobia, and difficulties obtaining a satisfactory supine position within the scanner, particularly in very obese or kyphotic patients.

2.2.3.4 Data Storage

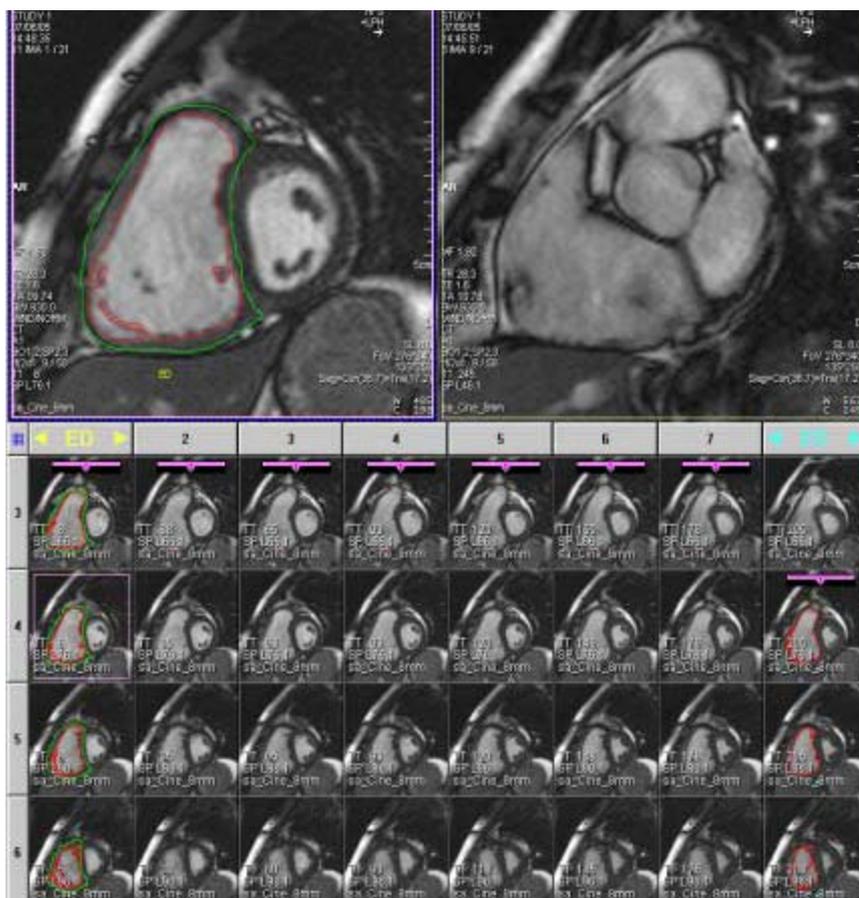
On completion of each CMR scan, the images were saved to the hard-drive of the scanner, and backed up onto a compact disc (CD). Individual CDs were coded by number and stored in a locked office until data analysis.

2.2.3.5 Analysis of Cardiovascular Magnetic Resonance Images

All CMR images in this thesis were analysed on a satellite workstation attached to the main MR scanner using the Argus analysis software (Siemens, Erlangen, Germany). Individual CDs, which corresponded to individual patients undergoing diagnostic assessment, were retrieved and analysed prior to right heart catheterisation. CMR scans performed prior to August 2008 were analysed in a similar fashion by previous SPVU research fellows (Dr Kevin Blyth, Dr Lindsey McLure).

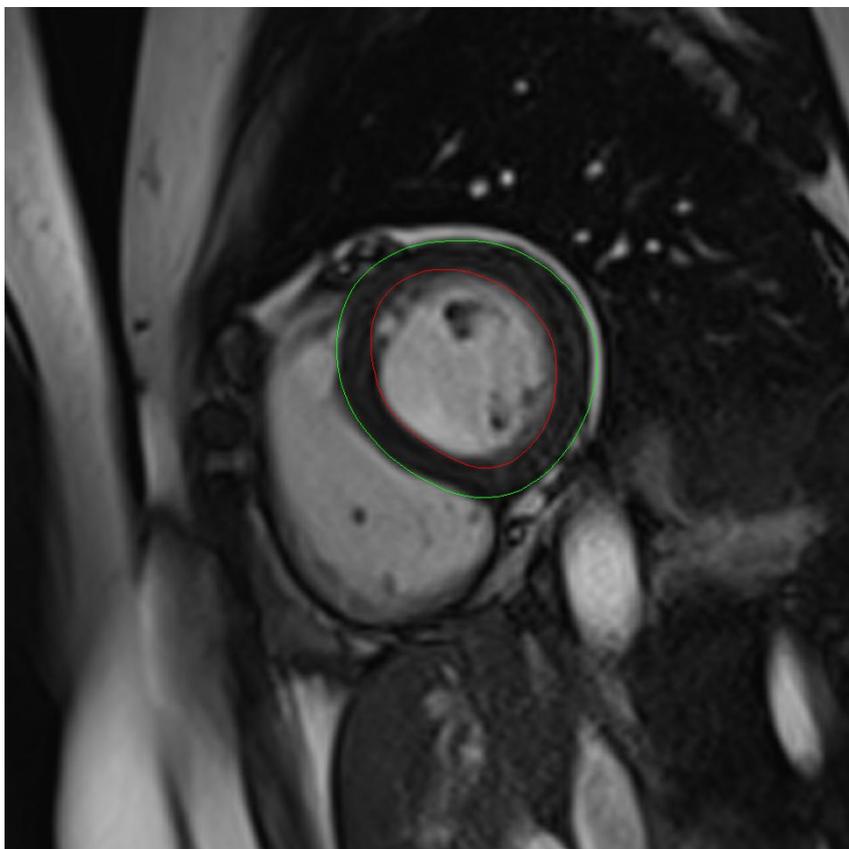
The standard technique of manual planimetry was used for analysing the CMR images. After loading the patient CD, the Argus programme automatically identified the images within each SA cine loop that had the largest and smallest blood volumes and defined these as end-diastole and end-systole respectively. The end-diastolic image was usually the first image acquired after R wave deflection. Using a cursor the epicardial and endocardial borders of the end-diastolic and end-systolic images at each slice position within the SA stack were manually defined. Trabeculations and papillary muscles were included in the analyses. An example of planimetric analysis of the right ventricle is displayed in Figure 2.6, and of the left ventricle in Figure 2.7.

Figure 2.6 An example of planimetry analysis of RV volumes and mass using the Argus analysis software.



Each row of short axis images represents a loop of cine images acquired during one cardiac cycle at consecutive slice positions, beginning at the base of the heart, moving apically to cover both ventricles. The first images within each row (or slice position) were acquired immediately after R-wave deflection. They are defined by the Argus software as the end-diastolic images (indicated by the yellow ED at the top of the left-hand column) and have the greatest total ventricular blood volume. The end-systolic images were defined automatically by the Argus software as the column of images with the smallest total ventricular blood volume, however this was also verified visually. A trackball mouse and cursor was used to define the endocardial (in red) and epicardial (in green) surfaces of the RV at end-diastole, and the endocardial surface only at end-systole (in red). This allowed calculation of RV end-diastolic and end-systolic volumes and RV mass.

Figure 2.7 Planimetric analysis of a left ventricular short axis image in a patient without PH



An example of planimetric analysis of the left ventricle is displayed on a short axis image from a patient who did not have pulmonary hypertension at RHC. A trackball mouse and cursor was again used to define the endocardial (in red) and epicardial (in green) surfaces of the LV.

The individual slice areas were multiplied by slice thickness (8mm) plus the inter-slice gap (2mm), Simpson's rule was applied, and the Argus software automatically calculated RVEDV, RVESV, LVEDV and LV end-systolic volume (LVESV). Stroke volumes for the RV and LV were determined as (RV/LV) EDV-ESV. Right and left ventricular ejection fractions were determined as a percentage (%) as:
 $((RV \text{ or } LV) SV/EDV) \times 100$.

In keeping with current CMR practice, RV and LV mass (RVM and LVM) were determined as the product of the difference between the end-diastolic and end-systolic volume for each ventricle and the density of cardiac muscle (1.05 g/cm^3). RV mass was determined as RV free wall mass, with the Interventricular Septum (IVS) considered part of the LV. Ventricular Mass Index (VMI) was determined as RVM/LVM, as previously shown¹²². Throughout this thesis, all ventricular volumes are corrected for Body Surface Area (BSA) and reported as indexed measurements: (RVEDV Index (RVEDVI), RVSV Index, (RVSVI), LVEDV Index (LVEDVI) and LVSV Index (LVSVI)).

2.2.3.6 Measurement of Atrial volumes

The biplane area length method was used to measure left atrial volume (LAV)²³⁵. The vertical and horizontal long axis cines were used to obtain images of the left atrium at maximal filling (Figure 2.8.). The atrial lengths and areas were measured from both views using the Argus image measurement tools. The left atrial endocardial area was manually traced to exclude the atrial appendage and pulmonary veins. The long-axis length of the left atrium was defined as the distance from the central of the mitral annulus to the posterior atrial wall. LAV was calculated using the equation:

Left Atrial Volume (LAV)

$$= (0.848 \times \text{area}_{4\text{chamber}} \times \text{area}_{2\text{chamber}}) / [(\text{length}_{4\text{ch}} + \text{length}_{2\text{ch}}) / 2]$$

LAV was corrected for body surface area (LAV/BSA).

Right atrial volume (RAV) was determined using the horizontal long axis cine image of the right atrium at maximal filling, using the monoplane area-length formula:

$$\text{Right Atrial Volume (RAV)} = 0.848 \times (\text{area}_{4\text{ch}})^2 / \text{length}_{4\text{ch}}$$

Figure 2.8 Vertical and horizontal long axis cine images used for calculating atrial volumes



The red shading is the left atrial area, the blue shading is the right atrial area, and the white arrows are the atrial lengths.

Modified from Whitlock *et al.* Comparison of left and right atrial volume by echocardiography versus cardiac magnetic resonance imaging using the area-length method. *American Journal of Cardiology*. 2010;106:1345-1350

2.3 Diagnosis and Commencement of PAH Treatment

2.3.1 Multi-Disciplinary Team Meeting

A multi-disciplinary team meeting (MDTM) was held on the Thursday afternoon of the diagnostic admission. In attendance were: The Pulmonary Vascular Research Fellow and Consultant, Consultant Radiologist, Consultant Cardiologist, Pulmonary Vascular Clinical Nurse Specialist, Clinical Trials Nurse. For each patient undergoing assessment the relevant clinical history, examination findings, imaging, non-invasive investigations and pulmonary haemodynamics were reviewed. A diagnosis was then made in accordance with current PH guidelines², and agreed by the MDTM.

2.3.2 Starting PAH-specific Therapy

If a diagnosis of PAH was made then the patient was offered PAH-specific therapy, in accordance with the current clinical guidelines²

If parenteral prostanoid treatment was being commenced then the patient was transferred to the Respiratory Unit (Wards 6C/7C) at Gartnavel General Hospital. Oral PAH therapy (PDE-5 Inhibitor, Endothelin Receptor Antagonist, Calcium Channel Blocker) was either commenced at the end of the diagnostic admission, or as an outpatient within 7 days of right heart catheterisation.

Continuous intravenous Epoprostenol was initially administered via a 2F peripherally inserted central catheter (PICC), before switching to a permanent hickmann line. The starting dose was typically 2ng/kg/min and was gradually uptitrated over a two-week period, dependent on patient tolerance and side-effects such as hypotension.

Inhaled Iloprost was administered via the I-neb Adaptive Aerosol Delivery (AAD) System. The starting dose was 2.5mcg per inhalation, with target of 6 – 9 inhalations per day.

The typical starting doses of oral PAH therapies were: Sildenafil 20mg tid, Ambrisentan 5mg od, Bosentan 62.5mg bd.

Patients receiving PAH-specific therapy in the context of a clinical trial were commenced on treatment in accordance with the relevant study protocol.

2.3.3 Follow-up after commencing PAH therapy

2.3.3.1 Out-patient Clinic

If disease-targeted PAH therapy was commenced following the diagnostic admission, then the patient was reviewed in the outpatient clinic after approximately 3-4 months, 6 months, 9 months and 12 months of treatment. Patients recruited into clinical trials were followed up as per the individual study protocol. Out-patient clinic visits were at Gartnavel General Hospital (prior to August 2008) or the Golden Jubilee National Hospital (from August 2008 onwards)

2.3.3.2 Assessment at Out-patient follow-up visits

Follow-up assessment involved clinical examination, documentation of NYHA functional class, and a repeat six-minute walk test was performed. Liver Function Tests and Full Blood Count were performed for patients receiving endothelin receptor antagonists, and where possible NT-ProBNP was performed at each visit.

2.3.3.3 Repeat Cardiac Magnetic Resonance Imaging

Patients recruited into the EURO-MR study underwent a repeat CMR scan after approximately 3-4 months of treatment, and again after approximately 12 months of treatment. The CMR protocol for these repeat scans was identical to the baseline CMR. Analysis of follow-up CMR images was performed immediately, and without knowledge of the follow-up 6MWT result and NYHA functional class.

2.3.3.4 Changes to PAH Therapy

At each out-patient visit the effectiveness of the PAH therapy that had been commenced at the diagnostic admission was assessed.

PAH therapy was then: continued at the current dose; continued at an increased dose (e.g. Sildenafil increased to 50mg tid); switched to a different class of drug (e.g. Sildenafil switched to Bosentan); or another drug was added to the initial therapy (e.g. Nebulized Iloprost added to Sildenafil). All changes to PAH therapy were discussed with a Consultant within the Scottish Pulmonary Vascular Unit.

2.4 Survival analysis

2.4.1 Cox Proportional Hazards Analysis

Cox proportional hazards model with right censoring was used to identify predictors of all-cause mortality. Survival time was calculated from the date of diagnostic RHC to censor date or date of death. Patients were censored at the time of data cut-off, lung transplantation or last clinical contact if lost to follow-up.

Univariate analysis was first performed to identify predictors of mortality from candidate variables. These variables were tested for collinearity using Pearson or Spearman correlation coefficients depending on data distribution. Variables with p value less than a predefined level in the univariate analysis or those considered clinically relevant based on previous studies were analysed in a multivariate Cox proportional model using a backward selection procedure. Highly correlated variables ($r > 0.7$) were not included. To avoid over-fitting, one covariate was included in the final model for approximately every ten deaths. A p value <0.05 by likelihood ratio test was defined as statistically significant.

2.4.2 Kaplan Meier Analysis

Kaplan Meier analysis was used to estimate survival rates using all-cause mortality as the end-point. Log-rank test was used for group comparison. Survival time was calculated from the date of diagnostic RHC to censor date or date of death. Patients were censored at the time of data cut-off, lung transplantation or last clinical contact if lost to follow-up. Results were presented as Kaplan Meier survival curves and the number of subjects at risk at regular time intervals. A p value <0.05 was defined as statistically significant.

2.4.3 Receiver Operating Characteristics Analysis

Receiver-operating characteristics (ROC) analysis was used to determine the discriminatory power and optimal threshold of a continuous variable in predicting all-cause mortality at a defined time point. A ROC curve was constructed by plotting sensitivity (y axis) against 1-specificity (x axis). The optimal threshold was identified from the curve by locating the point closest to the coordinate $y=1$ (100% sensitivity), $x=0$ (100% specificity).

The discriminatory power of a predictor variable was determined by calculating the area under the ROC curve (AUC). An AUC of 0.5 indicated that the variable had no discriminatory power whereas an AUC of 1 indicated that the variable had perfect discriminatory power.

Additional statistical methodology pertaining to each study in this thesis is described separately, within the relevant chapters.

Chapter 3

Left Atrial Volume by Cardiac Magnetic Resonance
Distinguishes Idiopathic Pulmonary Arterial Hypertension
From Pulmonary Hypertension due to Heart Failure with
Preserved Ejection Fraction

3.1 Introduction

3.1.1 Current methods for distinguishing IPAH from PH-HFpEF

As discussed in earlier chapters, current guidelines on the classification, diagnosis and management of pulmonary hypertension emphasise the importance of distinguishing IPAH from PH-HFpEF^{2, 4, 236}. It can be difficult to distinguish IPAH from PH-HFpEF on clinical grounds, particularly in older patients with significant medical comorbidities. Typically, compared with IPAH, patients with PH-HFpEF are older, more often female, and more frequently have other cardiovascular comorbidities including hypertension, diabetes, obesity, and coronary artery disease⁵⁷. In their cross-sectional study that included 522 patients with PAH and 100 patients with PH-HFpEF, Thenappan *et al* demonstrated that a model consisting of ten clinical characteristics (Age, WHO functional class, hypertension, obesity, diabetes mellitus, coronary artery disease, serum creatinine, diuretic use, beta-blocker use, and ACE inhibitors / Angiotensin Receptor Blocker use) could distinguish between the two conditions with an area under the ROC curve of 0.92⁵⁷.

Echocardiographic abnormalities typically observed in PH-HFpEF but not in PAH include left ventricular hypertrophy, grade II or grade III diastolic dysfunction, left atrial enlargement, and less frequent midsystolic notching pattern on the RV outflow tract Doppler signal¹⁹⁵. Robbins *et al* found that left atrial dilatation on echocardiography was present in 77% of patients with pulmonary venous hypertension but only 10% of patients with pulmonary arterial hypertension²²⁹. Similarly, Thenappan *et al* noted echocardiographic left atrial enlargement in 64% of their PH-HFpEF group and only 18% of the PAH group, however the addition of echocardiographic predictors (left ventricular posterior wall thickness, left atrial enlargement, and right atrial enlargement) to the model described above did not provide any significant incremental value over baseline clinical characteristics alone⁵⁷.

Right heart catheterisation is the definitive diagnostic technique for distinguishing IPAH from PH-HFpEF. At RHC, patients with PH-HFpEF typically have only a moderate increase in PAP and PVR, and higher cardiac output and aortic systolic pressures than IPAH patients. The key measurement that distinguishes the two conditions is the PAWP, with a PAWP of ≤ 15 mmHg excluding a diagnosis of PH-HFpEF². It is therefore essential that PAWP is measured accurately, ideally manually at end-expiration, rather than relying

on digitized results²³⁷. PAWP correlates closely with, although is generally slightly higher than, the mean left atrial pressure²³⁸. If an accurate wedge pressure measurement cannot be obtained, then left ventricular end-diastolic pressure (LVEDP) should be measured using left heart catheterisation¹⁹⁶. However, discrepancies between PAWP and LVEDP measurements are not uncommon, with some groups proposing that up to 50% of patients diagnosed with PAH based on a normal PAWP would be diagnosed with Group 2 PH if LVEDP measurements were used²³⁹.

When the clinical picture is in keeping with PH-HFpEF but the initial PAWP is normal (e.g. if patients are fasting and/or over-diuresed) then additional provocative measures such as fluid challenge or exercise may be performed during cardiac catheterisation. In a study of 207 patients with an initial haemodynamic profile of PAH at RHC, a 500ml infusion of normal saline over 5-10 minutes resulted in 46 patients (22.2%) developing a PAWP of >15mmHg and being reclassified as “occult pulmonary venous hypertension”¹⁰⁰. It is worth noting that: PAWP also significantly increased after fluid challenge in the “PAH” group (from 9 ± 3 mmHg to 11 ± 4 mmHg, $p < 0.001$)¹⁰⁰, PAWP has been reported to increase after acute saline loading in normal individuals²⁴⁰, and that there is no firm consensus on the volume of saline, rate of infusion, and the magnitude of increase in PAWP required for differentiating PH-HFpEF from PAH¹⁹⁶.

3.1.2 The Structure and Function of the Left Atrium

The left atrium is a thin-walled structure located in the inflow path from the pulmonary veins to the left ventricle (LV). Its anterior wall lies behind the transverse pericardial sinus, and its posterior wall is located close to the tracheal bifurcation and the oesophagus, and receives the pulmonary veins²⁴¹. It consists of (a) a main body with smooth walls, embryologically developed from the outgrowth of the pulmonary veins and (b) a trabeculated finger-like appendage.

The mechanical function of the left atrium can be defined by three phases of volume variation during the cardiac cycle. During ventricular systole the LA collects blood coming from the pulmonary veins, the “reservoir phase”. In this phase LV contraction and LA relaxation results in movement of the mitral annulus towards the cardiac apex, with an overall increase in LA volume²⁴². Atrial compliance allows chamber volume to increase during the reservoir phase, but preserving filling pressures within normal limits. At the end of ventricular systole, a reduction in atrial compliance leads to an increase in LA pressure, and in early diastole the high early diastolic atrial–ventricular pressure gradient results in blood stored in LA during the reservoir phase being driven into LV. Following this, direct flow of blood from the pulmonary vein through the atrium into the left ventricle occurs, the “conduit phase”. Finally there is a “contractile phase” during late diastole, when the LA muscle contracts in order to increase left ventricular stroke volume by approximately 20%²⁴¹. In early-stage LV filling impairment there is augmentation of LA reservoir and pump functions, however at end-stage ventricular dysfunction the limits of atrial preload reserve have been reached and therefore conduit of blood through the atrium will take precedence²⁴³.

A normal “compliant” left atrium can prevent elevations in LVEDP from resulting in damage to the pulmonary vasculature. By contrast, if the left atrium is stiff then post capillary PH can occur even in the setting of normal LVEDP²⁴⁴. Left atrial enlargement is not felt to be part of the normal ageing process, and maximum and minimum LA volumes do not differ between younger and older study populations^{245, 246}. Similarly, the impact of gender on LA volume is largely accounted for by the differences in body surface area between men and women^{247, 248}.

In PH-HFpEF there is preserved LV systolic function, i.e. normal LV ejection fraction, but impaired diastolic function with abnormal LV relaxation and increased LV filling pressure²⁴⁹. Sustained elevations of LV filling pressures can result in enlargement of the thin-walled left atrium, allowing left atrial volume to be used as a marker of the severity and chronicity of diastolic dysfunction^{206, 250-253}. Left atrial volume is higher in patients with HFpEF than in asymptomatic hypertensive patients or healthy controls²⁵⁴. In patients with HFpEF, LAVI correlates with NT-pro-BNP levels in the setting of an elevated E/E' (early transmitral inflow to diastolic velocity of the mitral annulus) ratio at echocardiography²⁵⁵.

3.1.3 Assessment of Left Atrial Size

Under normal conditions, the left atrium may have a spherical or ellipsoid shape, however with even mild chamber distortion it enlarges asymmetrically due to the presence of surrounding structures and eccentrically directed mitral regurgitation jets. Therefore, LA volume is preferred over linear dimensions such as diameter or area, since it allows more accurate measurement of the asymmetric remodelling of the atrial chamber²⁵⁶.

There are a number of methods that can be used to determine left atrial volume with CMR. The most precise method is the multiple slice method (MSM) or Simpson's method, which involves time-consuming manual tracings of left atrial area on each cross-sectional image, and has been found to very closely relate to postmortal assessment of true LA size²⁵⁷. The biplane area-length method (ALM) is a much simpler method of assessing LAV. It uses the formula: $LAV = (0.848 \times \text{area}_{4\text{ch}} \times \text{area}_{2\text{ch}}) / [(\text{length}_{4\text{ch}} + \text{length}_{2\text{ch}}) / 2]$, where 4ch and 2ch is the 4-chamber and 2-chamber view respectively, and is usually then indexed to body surface area²⁵⁸. The ALM has been found to correlate closely with the MSM, with no significant difference in intraobserver and interobserver variability between the techniques²⁵⁹⁻²⁶². A third method, the prolate ellipse technique, consistently underestimates LAV compared with the other two methods²⁶³.

In their study of 85 patients and 18 controls, Whitlock *et al* demonstrated that atrial volumes estimated using the area-length method were significantly smaller when measured using echocardiography than when measured using CMR (LA volume 35 ± 20 vs 49 ± 30 ml/m², $p < 0.001$)²⁵⁸. In patients with permanent atrial fibrillation (AF), CMR by the area-length method can be used to assess left atrial size with excellent levels of intra-observer and inter-observer agreement²³⁵, superior to the reproducibility seen with 2D-transthoracic echocardiography²⁶⁴. In AF, left atrial volume measured by ALM at CMR has been found to have superior correlation to CMR-MSM than LAV measured by ALM at echocardiography²⁶⁵.

In a small study investigating the influence of CMR field strength on left atrial volumes and function, there was no significant difference in LAV in healthy volunteers between CMR at 1.5 Tesla and 3 Tesla²⁶⁶. Similarly, there was no difference in left atrial volume measured at end-diastole using prospective ECG triggering or retrospective gated sequences²⁶⁷.

There have been a number of studies performed to establish a normal reference range for left atrial volumes for healthy individuals with no history of cardiovascular disease. The most recent update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging (2015) has recommended that the upper normal limit for 2D echocardiographic LA volume is 34 ml/m² for both genders²⁶⁸, whilst the normal value using CMR is estimated to be 40 ml/m²²⁴⁸.

The experiment in this chapter tested the hypothesis that left atrial volume measured using CMR imaging would be able to distinguish IPAH from PH-HFpEF, and might be considered a non-invasive alternative to right heart catheterisation.

3.2 Methods

3.2.1 Patients

Consecutive treatment-naïve patients with a suspected new diagnosis of pulmonary hypertension were admitted for diagnostic assessment between January 2009 and March 2011 as described in Chapter 2.2. Patients underwent CMR imaging followed by RHC within 72 hours.

Patients with clear evidence of an underlying cause of pulmonary hypertension such as chronic thromboembolic disease, connective-tissue disease, left ventricular systolic dysfunction, or significant (\geq moderate) mitral or aortic valvular disease on echocardiography were excluded. In 58 patients the diagnostic issue that remained was whether the patient had IPAH or PH-HFpEF.

3.2.2 CMR Image Acquisition

CMR image acquisition was performed as described in Methods Section 2.2.3

3.2.3 CMR Image Analysis

CMR image analysis was performed as described in Methods Sections 2.2.3.5 and 2.2.3.6.

3.2.4 Statistical Analysis

A retrospective analysis of 40 CMR scans performed in our unit between 2005 and 2008 had shown that left atrial volume was higher in patients diagnosed with PH-HFpEF than those with IPAH (IPAH 20 ± 8 ml/m², PH-HFpEF 62 ± 15 ml/m², mean difference 41.8 ml/m²). A power calculation based on mean values and standard deviations from this analysis, aiming for a power of 90% and assuming a Type 1 error of 0.05, had suggested that only 4 patients in each group were needed to demonstrate significant differences in left atrial volume if present.

For all variables, normal Gaussian distribution was verified using histograms and Kolmogorov-Smirnov tests. For demographic, CMR, and haemodynamic variables mean values \pm one standard deviation (\pm SD) were calculated. Categorical variables are

presented as number (%). Student's t-test was used to compare the mean values between the groups. The Chi-squared test was used to compare categorical variables. Correlations between normally distributed CMR and haemodynamic variables were tested by Pearson's method. ROC analysis was used to determine the respective AUC for non-invasive variables that could be used to distinguish IPAH from PH-HFpEF. Optimal thresholds were identified by selecting the data point closest to the coordinate $y=1$ (100% sensitivity), $x=0$ (100% specificity) on the ROC curves. No adjustment was made to P-values to account for multiple testing. A p value < 0.05 was defined as statistically significant.

All calculations were performed using GRAPHPAD Prism (Version 5.00; GraphPad Software Inc, La Jolla, CA, USA).

3.3 Results

3.3.1 Clinical characteristics

Clinical characteristics for the study population are displayed in Table 3.1.

Table 3.1 Clinical characteristics

	IPAH (n = 37)	PH-HFpEF (n = 21)	P-value
Age (years)	60 (\pm 15)	71 (\pm 12)	0.004
Sex (♀; ♂)	19 ; 18	14 ; 7	
Functional Class (II; III; IV)	9 ; 26 ; 2	8 ; 13 ; 0	
Body Surface Area (m ²)	1.79 (\pm 0.21)	1.84 (\pm 0.28)	0.40
Diabetes Mellitus, n (%)	7 (19)	9 (43)	0.0029†
Atrial Fibrillation, n (%)	1 (3)	16 (76)	< 0.0001†
Systolic BP (mmHg)	127 (\pm 24)	161 (\pm 26)	< 0.0001
Diastolic BP (mmHg)	79 (\pm 12)	81 (\pm 13)	0.49
Mean BP (mmHg)	97 (\pm 13)	111 (\pm 14)	0.0004
Resting Heart Rate (bpm)	76 (\pm 14)	75 (\pm 18)	0.87
6MWD (m)	261 (\pm 144)	251 (\pm 100)	0.80
Serum Creatinine (μ mol/L)	94 (\pm 31)	115 (\pm 44)	0.03

IPAH, idiopathic pulmonary arterial hypertension; PH-HFpEF, pulmonary hypertension associated with heart failure with preserved ejection fraction; 6MWD, six-minute walk distance

Data presented as mean (\pm SD) otherwise specified

† - using the Chi-squared test for categorical variables

The IPAH group was younger, had a higher proportion of males, and had lower systemic blood pressure than the PH-HFpEF group. There was a higher incidence of diabetes mellitus and atrial fibrillation in the PH-HFpEF group. There was no significant difference between the groups in body surface area, resting heart rate or distance achieved on the six-minute walk test. The PH-HFpEF group had higher serum creatinine than the IPAH group (115 ± 44 vs 94 ± 31 $\mu\text{mol/L}$, $p = 0.03$).

At echocardiography, the IPAH group had a significantly lower left atrial area than the PH-HFpEF group (14 ± 4 vs 25 ± 5 cm^2 , $p < 0.0001$), however left atrial area could not be assessed in 10 of the 58 study patients (17%) due to poor image quality. Other echocardiographic measurements of left ventricular diastolic function (E/A ratio, E/E' ratio) were not routinely performed during the diagnostic assessment.

3.3.2 Pulmonary haemodynamics

Invasive measurements obtained in the IPAH and PH-HFpEF groups at right heart catheterisation are shown in Table 3.2. The IPAH group had lower PAWP, lower cardiac output and higher PVR than the PH-HFpEF group. There was, however, no significant difference in systolic or mean PAP between the groups.

Table 3.2 Pulmonary haemodynamics at right heart catheterisation

	IPAH (n = 37)	PH-HFpEF (n = 21)	P-value
Systolic PAP (mmHg)	80 (\pm 14)	77 (\pm 13)	0.51
Diastolic PAP (mmHg)	30 (\pm 8)	24 (\pm 8)	0.008
Mean PAP (mmHg)	48 (\pm 9)	44 (\pm 9)	0.17
PAWP (mmHg)	7 (\pm 4)	23 (\pm 6)	<0.0001
Cardiac Output (L/min)	3.7 (\pm 1.4)	4.6 (\pm 1.3)	0.03
PVR (Wood Units)	12.5 (\pm 5.5)	5.0 (\pm 2.2)	<0.0001
RAP (mmHg)	8 (\pm 6)	14 (\pm 5)	0.0007

IPAH, idiopathic pulmonary arterial hypertension; PH-HFpEF, pulmonary hypertension associated with heart failure with preserved ejection fraction; PAP, pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; DPAP, diastolic pulmonary artery pressure; PVR, pulmonary artery pressure; RAP, right atrial pressure.

Data presented as mean (\pm SD) otherwise specified

3.3.3 Cardiac magnetic resonance imaging

Ventricular dimensions, systolic function and atrial volumes for the groups are presented in Table 3.3.

Table 3.3 CMR Results in patients with IPAH and PH-HFpEF

	IPAH (n = 37)	PH-HFpEF (n = 21)	P-value
Right Ventricle			
EDV Index (ml/m ²)	88 (± 25)	72 (± 26)	0.023
Stroke Volume Index (ml/m ²)	26 (± 9)	31 (± 11)	0.04
Ejection Fraction (%)	31 (± 13)	45 (± 11)	0.0001
Mass (g/m ²)	58 (± 13)	45 (± 15)	0.002
Left Ventricle			
EDV Index (ml/m ²)	42 (± 13)	60 (± 22)	0.0002
Stroke Volume Index (ml/m ²)	26 (± 9)	36 (± 13)	0.002
Ejection Fraction (%)	64 (± 14)	63 (± 16)	0.82
Mass (g/m ²)	49 (± 9)	62 (± 21)	0.002
Atrial Analysis			
Right Atrial Volume (ml/m ²)	62 (± 27)	61 (± 43)	0.93
Left Atrial Volume (ml/m ²)	24 (± 9)	67 (± 20)	<0.0001

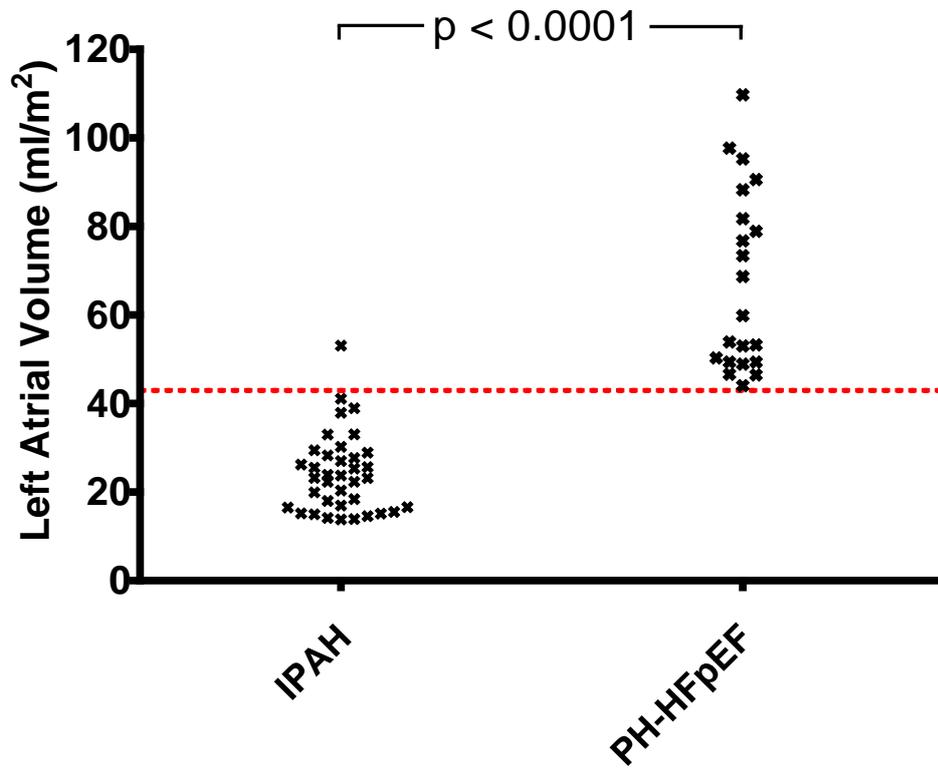
IPAH, idiopathic pulmonary arterial hypertension; PH-HFpEF, pulmonary hypertension associated with heart failure with preserved ejection fraction; EDV, end-diastolic volume.

Data presented as mean (±SD) otherwise specified

In IPAH the right ventricle was more dilated and hypertrophied (increased RVEDV and RV mass) than in PH-HFpEF, but had poorer systolic function (reduced RVEF and RV stroke volume). In IPAH the left ventricle was poorly filled (reduced LVEDV and LV stroke volume). Left ventricular systolic function was found to be well preserved in both groups.

Right atrial volume was increased in both IPAH and PH-HFpEF (62 ± 27 vs 61 ± 43 ml/m², $p = 0.93$). Left atrial volume was significantly lower in IPAH than in PH-HFpEF (24 ± 9 vs 67 ± 20 ml/m², $p < 0.0001$) (Figure 3.1.).

Figure 3.1 Left atrial volume in IPAH versus PH-HFpEF



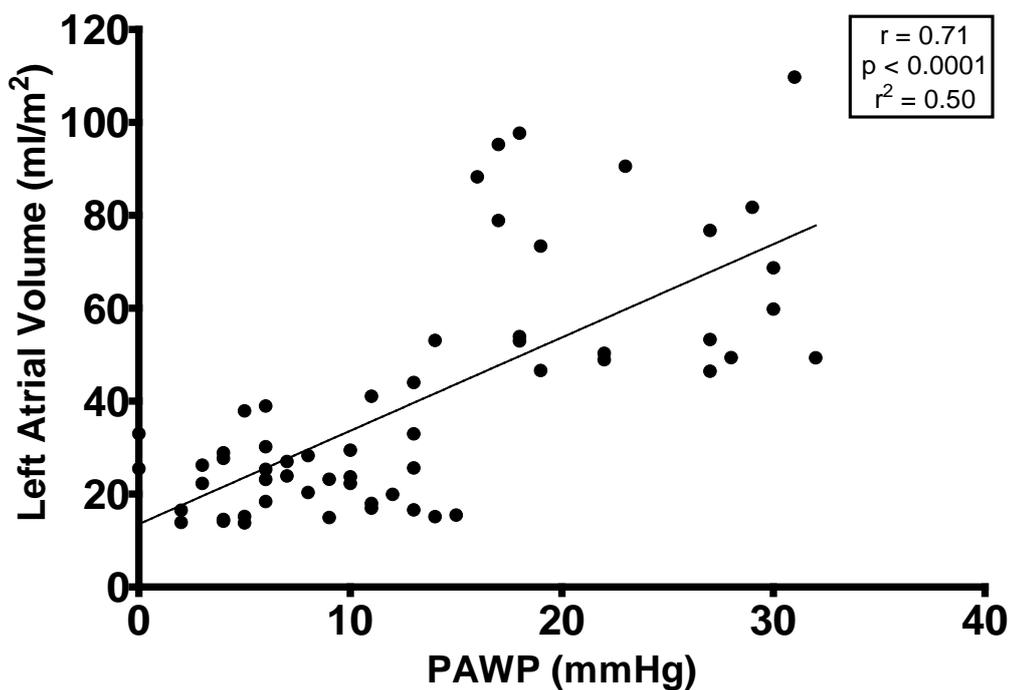
Left Atrial Volume (LAV) calculated from cardiac magnetic resonance was significantly lower in idiopathic pulmonary arterial hypertension (IPAH) than in pulmonary hypertension associated with heart failure with preserved ejection fraction (PH-HFpEF).

----- represents a LAV threshold of 43ml/m²

When the PH-HFpEF group was stratified by the median PVR, there was no difference in left atrial volume between patients with PH-HFpEF and supramedian PVR ($PVR \geq 5.0$) and inframedian PVR ($PVR < 5.0$) (63 ± 18 vs 73 ± 22 ml/m², $p = 0.27$). Similarly, when the PH-HFpEF group was stratified by the diastolic pulmonary gradient (DPG), 17 patients had “isolated postcapillary hypertension” (i.e. mean PAP > 25 mm Hg, PAWP > 15 mm Hg, and DPG < 7 mm Hg) and 4 of 21 had “combined postcapillary and precapillary PH” (i.e. mean PAP > 25 mm Hg, PAWP > 15 mm Hg, and DPG ≥ 7 mmHg), however there was no difference in LAV between these groups (68 ± 21 vs 64 ± 22 ml/m², $p = 0.69$).

There was strong correlation between left atrial volume at CMR and PAWP measured at right heart catheterisation ($r = 0.71$, $p < 0.0001$) (Figure 3.2).

Figure 3.2 Correlation between PAWP measured at right heart catheterisation and left atrial volume at CMR

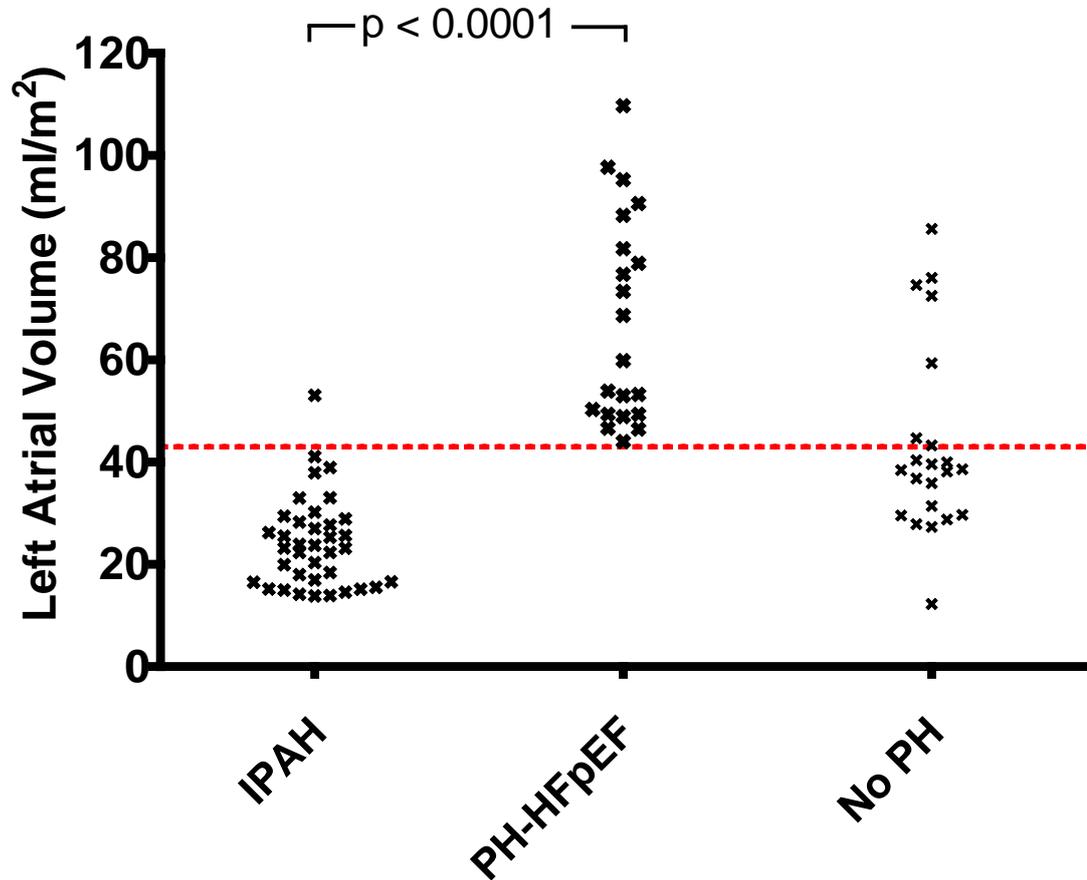


Correlation between PAWP obtained at right heart catheterisation and LAV measured using CMR, in 37 patients diagnosed with IPAH and 21 with PH-HFpEF. LAV correlated strongly with PAWP.

PAWP, pulmonary artery wedge pressure; LAV, left atrial volume; IPAH, idiopathic pulmonary arterial hypertension; PH-HFpEF, pulmonary hypertension due to heart failure with preserved ejection fraction

An additional twenty-three patients underwent CMR assessment who did not have pulmonary hypertension at right heart catheterisation (i.e. mPAP < 25 mmHg). LAV for this group was $49 \pm 33 \text{ ml/m}^2$ (Figure 3.3.)

Figure 3.3 Left atrial volume in IPAH, PH-HFpEF and patients with no PH



IPAH, idiopathic pulmonary arterial hypertension; PH-HF-pEF, pulmonary hypertension due to heart failure with preserved ejection fraction; PH, pulmonary hypertension

3.3.4 ROC Analysis

Receiver operating characteristic (ROC) analysis was performed on the non-invasive variables which could potentially distinguish IPAH from PH-HFpEF. These included age, systolic BP, serum creatinine, RV ejection fraction, and left atrial volume (Table 3.4).

Table 3.4 ROC Analysis of non-invasive variables to distinguish IPAH from PH-HFpEF

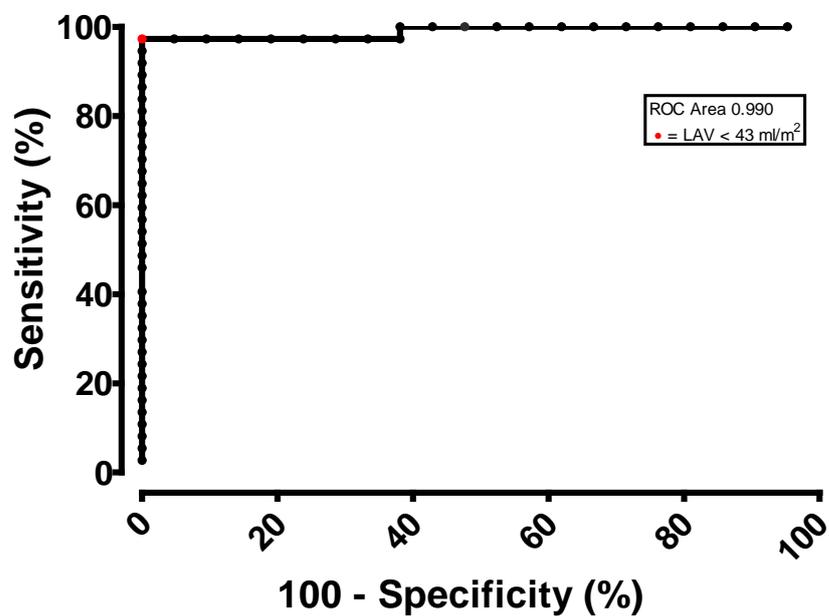
Variable	Criterion*	AUC	P Value
Serum Creatinine ($\mu\text{mol/L}$)	< 96	0.685	0.020
Age (yrs)	< 62	0.734	0.003
RV Ejection Fraction (%)	< 38	0.801	0.0002
Systolic BP (mmHg)	< 132	0.839	<0.0001
CMR Left Atrial Volume (ml/m^2)	< 43	0.990	<0.0001

AUC, area under the curve; ROC, receiver operating characteristic curve; IPAH, idiopathic pulmonary arterial hypertension; PH-HFpEF, pulmonary hypertension associated with heart failure with preserved ejection fraction; BP, blood pressure; RV, right ventricle.

* The criterion value presented had the optimal balanced sensitivity and specificity for distinguishing IPAH from PH-HFpEF

Left atrial volume was found to be the most sensitive and most specific single variable, with an area under the ROC curve of 0.990 (Figure 3.4). Using a threshold LAV of less than 43 ml/m^2 , IPAH could be distinguished from PH-HFpEF with 97% sensitivity and 100% specificity.

Figure 3.4 ROC curve of left atrial volume for distinguishing IPAH from PH-HFpEF



Receiver operating characteristic (ROC) curve demonstrating a left atrial volume threshold of less than 43ml/m^2 is a highly sensitive and specific way to distinguish idiopathic pulmonary arterial hypertension (IPAH) from pulmonary hypertension associated with heart failure with preserved ejection fraction (PH-HFpEF).

3.4 Discussion

This chapter has identified that CMR-derived left atrial volume is a sensitive and specific way to distinguish IPAH from PH-HFpEF. At present, IPAH is distinguished from PH-HFpEF by demonstration of a PAWP of ≤ 15 mmHg at right heart catheterisation, however a left atrial volume threshold of < 43 ml/m² at CMR could be considered as a simple, non-invasive alternative. Left atrial volume at CMR appears to correlate closely with invasive PAWP, and in the setting of preserved LV systolic function an elevated LAV will reflect LV diastolic dysfunction.

Historically IPAH was considered to be a disease that predominately affected young females, however recent epidemiological studies have demonstrated that patients of any age can be affected, and there is growing awareness of the prevalence of IPAH in the elderly population^{81, 184, 269}. Traditionally, patients with PH-HFpEF were managed solely in the general cardiology clinic rather than at a specialist tertiary PH centre. However, PH centres are now spending an increasing amount of time and resources distinguishing IPAH from PH-HFpEF²⁷⁰. The importance of distinguishing IPAH from PH-HFpEF has increased over the last two decades following the growth in therapeutic options now available for IPAH. These new therapies have been shown to improve symptoms, increase exercise capacity, delay time to clinical worsening, and in the case of intravenous prostacyclin, improve survival in IPAH^{53, 151, 271, 272}. However, PAH-specific therapies are very expensive, and are not effective but instead may be potentially harmful in PH-HFpEF.

The current study has demonstrated that a single non-invasive variable, CMR-derived left atrial volume, can be used to distinguish accurately IPAH from PH-HFpEF. This is in contrast to other published models that have used a combination of clinical, biochemical, haemodynamic and imaging criteria to distinguish between these conditions^{57, 273}. It is worth noting that in the study by *Thenappan et al* the majority of patients in the PAH group did not have idiopathic PAH, but instead had PAH associated with connective-tissue disease, congenital heart disease or portal hypertension⁵⁷. In clinical practice, the most important diagnostic uncertainty is distinguishing IPAH from PH-HFpEF.

Left atrial size is already an established marker of cardiovascular disease. Increased left atrial size at echocardiography predicts adverse cardiovascular events^{274, 275}, with left atrial volume being a superior predictor to area or diameter²⁷⁶. Increased LAV at CMR was

associated with poorer survival in patients with end-stage renal disease and left ventricular hypertrophy²⁷⁷. Similarly, LAV was higher in patients with stage 3 and 4 chronic kidney disease than in those with normal renal function, and in these patients an increased LAV was predictive of adverse cardiac events²⁷⁸. Abnormalities in left atrial structure and function, including an elevated LAV, have been shown to precede development of heart failure symptoms in a group of patients previously free of clinical cardiovascular diseases²⁷⁹. LAV predicts heart failure hospitalization and mortality with similar statistical power as LV ejection fraction in adults with coronary artery disease²⁸⁰. LAV at CMR was the main determinant of outcome following pulmonary vein isolation plus linear lesion ablation for paroxysmal-persistent atrial fibrillation²⁸¹.

Patients with IPAH were found to have a lower LAV than is reported for healthy controls²⁵⁸. The left heart is poorly filled in IPAH as impaired right ventricular systolic function, reduced RV stroke volume and progressive luminal obliteration of small pulmonary arteries results in a reduced volume of blood reaching the left atrium. Indeed, it has been shown that a low LV end-diastolic volume, a marker of poor LV filling, is associated with increased mortality in IPAH¹¹⁸.

In this study CMR was used as the main imaging modality to assess left atrial size rather than echocardiography or CT. Whilst this may limit the applicability of the current results to specialist centres with CMR facilities, it has been demonstrated previously that echocardiography significantly underestimates CMR-derived LAV, in both healthy subjects and patients with cardiovascular disease²⁵⁸. This is thought to be the result of poorer endocardial definition and less accurate delineation of pulmonary veins and atrial appendages²⁸². CMR is already considered the ‘gold standard’ for evaluating the right ventricle in pulmonary hypertension, providing valuable information about right ventricular morphology and function¹⁰⁹. Left atrial volume assessment was performed by a single operator, however this technique has been shown to have low inter-observer and intra-observer variability. Echocardiographic assessment of left atrial size could potentially be useful in distinguishing IPAH from PH-HFpEF in non-specialist centres, however it should be noted that this could not be performed in 17% of patients in the current study. The findings of the current study suggest that LAV measured with CMR may be a useful method for distinguishing IPAH from PH-HFpEF, however given the small number of patients it not possible to assess the added value of CMR over clinical and echocardiographic data.

In the current study a prospective methodology (i.e. using previously established CMR cut-offs to establish diagnosis and then confirming these using RHC) was not used. This was due to the absence of an established ‘normal’ range for CMR left atrial volume in IPAH, and the relatively small number of patients in our retrospective pre-2008 analysis. It is also important to acknowledge that in the current study the precise diagnosis was not fully established to current standards, with no standardized protocol for the use of fluid challenge or exercise during RHC, and no patients subsequently underwent measurement of LVEDP. The importance of fully establishing diagnosis was highlighted in the recent AMBITION study investigating ERA and PDE-5 combination therapy versus monotherapy, where approximately 60% of the first 100 patients recruited to the study had a “left heart” profile (older age, AF, hypertension, diabetes) despite a PAWP ≤ 15 mmHg²⁸³. This recognition of the high prevalence of risk factors for LV diastolic dysfunction resulted in a change to the eligibility criteria for the study, with the exclusion of patients with three or more risk factors for LV diastolic dysfunction, and a tightening of haemodynamic entry requirements.

3.5 Conclusions

One of the most important diagnostic challenges for a pulmonary vascular centre is to distinguish IPAH from PH-HFpEF, particularly since the emergence of PAH-specific therapies. This study has demonstrated that a single, noninvasive, CMR-derived variable, LAV of $<43 \text{ ml/m}^2$, can distinguish these two conditions with high sensitivity and specificity. At present, a normal PAWP measured at RHC is currently used to distinguish IPAH from PH-HFpEF. LAV measured by CMR correlates closely with PAWP, and future studies may help determine if it could be a suitable non-invasive alternative to cardiac catheterisation.

Chapter 4

Changes in Right Ventricular Function Measured by
Cardiac Magnetic Resonance Imaging in Patients Receiving
Pulmonary Arterial Hypertension–Targeted Therapy

4.1 Introduction

In Chapter 3 it was shown that left atrial volume measured by CMR could be used to distinguish idiopathic PAH from PH due to HFpEF, and therefore identify which patients would be suitable to receive disease-targeted PAH therapy. In PAH, increased pressure in the pulmonary arteries overloads the right ventricle (RV), causing hypertrophy and eventually RV failure^{23, 284, 285}. Most measures that are known to predict survival in PH relate directly to RV function (right atrial pressure, cardiac index, TAPSE) or correlate with measures related to RV function (e.g. exercise capacity and functional class)^{1, 69, 230, 231}. However, whilst the past two decades have seen the introduction of several treatments targeting the pathophysiological mechanisms of PAH, these agents have been approved largely based on improved haemodynamic variables or exercise capacity. Consequently, disease progression and treatment efficacy have typically been assessed using 6MWD or RHC (in non-UK centres). Recent interest in the RV and its response to change in afterload has raised the possibility that direct assessment of RV function may be a more appropriate way of determining response to therapy and monitoring disease progression^{38, 286}.

4.1.1 Disease targeted PAH therapy and exercise capacity

The 6MWT has been used as a primary outcome measure in many clinical trials of PAH therapy due to its simplicity, low cost and reproducibility. In the pivotal trials of PAH monotherapy, absolute improvements in 6MWD have ranged from -10m to +108m, but have usually been around 30m to 45m (Table 4.1). Clinical trials investigating the use of combination PAH therapy have noted less impressive improvements in placebo-adjusted 6MWD, despite improvements in pulmonary haemodynamics and rates of clinical worsening²⁸⁷. In the EARLY (Endothelin Antagonist tRial in mildLY symptomatic pulmonary arterial hypertension) trial, six months of bosentan treatment in WHO FC II patients had no significant effect on 6MWD despite reducing PVR and improving TTCW⁷⁹. A similar result has been demonstrated in patients with well preserved 6MWD (>450m) at diagnosis, in that disease targeted therapy improved functional class and cardiac index but 6MWD remained unchanged⁸⁰.

Pooled data from 10 randomized trials of PAH therapy (including 2404 patients) was used to investigate whether changes in 6MWD after 12 weeks of treatment could predict the relationship between treatment assignment (active vs placebo) and occurrence of a predefined clinical event (death, transplantation, hospitalization for worsening PAH, need for additional PAH therapy). Whilst Delta 6MWD did meet the criteria for being a mediator of this relationship, the association was weak (22.1% of the treatment effect) and a large proportion of the treatment effect was not explained by changes in 6MWD²⁸⁸.

Table 4.1 Change in 6MWD in key PAH therapy trials

Author	Year	Pts	Active Drug	Study Period	Other Therapy	Aetiology	Δ 6MWD
Rubin ²⁸⁹	1990	23	Epoprostenol v Controls	8wks		IPAH	+45m
Barst ²⁷¹	1996	81	Epoprostenol v Controls	12wks		IPAH	+47m
Badesch ¹⁶⁷	2000	111	Epoprostenol v Controls	12wks		APAH	+108m
Channick ¹⁵¹	2001	32	Bosentan v Placebo	12wks		84% IPAH 16% APAH	+76m
Simmoneau ¹⁴⁸	2002	470	Treprostinil v Placebo	12wks		58% IPAH 42% IPAH	+16m
Olschewski ²⁹⁰	2002	203	Iloprost v Placebo	12wks		50% IPAH 22% APAH 28% CTEPH	+36m
Rubin ²⁹¹	2002	213	Bosentan v Placebo	16wks		70% IPAH 30% APAH	+44m
Humbert ²⁹²	2004	33	Bosentan vs Placebo	16wks	EPO	IPAH 82% APAH 18%	Epo & Bos +68m Epo & Placebo +74m
Galie ²⁷²	2005	278	Sildenafil v Placebo	12wks		IPAH 64% APAH 30%	+45m
Wilkins ²⁹³	2005	26	Bosentan vs Sildenafil	16wks		IPAH 88% APAH 12%	Both increased by +59m
McLaughlin ²⁹⁴	2006	67	Inhaled Iloprost vs Placebo	12wks	EPO	IPAH 55% APAH 45%	+26m
Hoepfer ²⁹⁵	2006	40	Inhaled Iloprost vs Placebo	12wks	Oral Bosentan	IPAH	-10m
Galie ²⁹⁶	2008	394	Ambrisentan vs Placebo	12wks		IPAH 64% APAH 32%	+45m
Galie ⁷⁹	2008	185	Bosentan vs Placebo	24wks		IPAH 61% APAH 35%	+19m
Simmoneau ²⁸⁷	2008	267	Sildenafil vs Placebo	16wks	EPO	IPAH 79% APAH 21%	+29m

EPO, epoprostenol; IPAH, idiopathic pulmonary arterial hypertension; APAH, associated pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension

4.1.2 Disease targeted PAH therapy and Survival

Epoprostenol is the only treatment that has been shown to improve survival in IPAH in a randomised controlled trial²⁷¹. In this landmark study 81 IPAH patients in functional class III and IV were randomised to intravenous epoprostenol plus conventional therapy or conventional therapy alone. After 12 weeks, the epoprostenol treated group had improved quality of life scores, improved functional class, increased 6MWD and improved haemodynamic parameters compared with the conventional group. Eight patients died during the 12 weeks study period, all of whom had been in the conventional therapy group. Based on this result intravenous epoprostenol is recommended as the first-line therapy for WHO FC IV PAH patients, and is used as a “rescue” therapy in the deteriorating patient²³². However, subsequent randomised placebo-controlled trials using mortality as an endpoint could no longer ethically be performed.

The impact of disease-targeted PAH therapy on survival has been investigated by means of meta-analysis. The meta-analysis of the 3 epoprostenol randomised controlled trials showed a relative risk reduction in mortality of 68-70%^{167, 271, 289}. In their meta-analysis of 23 randomised controlled trials of all classes of PAH therapy involving 3199 patients, *Galie et al* demonstrated that treatment with disease targeted therapies (after an average treatment period of 14.3 weeks) improved survival in PAH, with 43% reduction in mortality and 61% reduction in hospital stays²⁹⁷.

Improved survival of PAH in the disease-targeted therapy era has also been demonstrated by comparing survival in the current era with that of historical controls. The NIH (National Institute of Health) registry was a multicentre, prospective registry from the pre-treatment era where 194 patients in the United States with the diagnosis of primary pulmonary hypertension were enrolled from between 1981 and 1985 and followed through to 1988⁵⁴. The median survival was 2.8 years after diagnosis, with estimated 1-, 3- and 5-year survival rates were 68%, 48% and 34% respectively¹. The recent REVEAL (Registry to Evaluate Early and Long-term PAH Disease Management) registry from the United States has demonstrated improved 1-, 3- and 5-year survival rates of 91%, 74% and 65% in the 1166 patients with idiopathic and heritable PAH²⁹⁸. Whilst the NIH and REVEAL registries included both incident and prevalent patients, in the UK study of a purely incident, treatment-naïve cohort of idiopathic, heritable, and anorexigen-associated PAH, patients diagnosed in 2001-2003 were more likely to have a shorter survival time compared with patients diagnosed in 2007–2009 ($p = 0.019$)¹⁸⁵. Survival in PAH

associated with systemic sclerosis has also improved in the disease-targeted therapy era, with one- and three- year survival rates now of 78 and 47%, compared with one year survival of 45% in a historical cohort^{299, 300}.

The experiment in this chapter tested the hypothesis that direct assessment of RV function using CMR would be an appropriate way of determining response to therapy and monitoring disease progression in PAH.

4.2 Methods

4.2.1 Patients

As part of the EU Framework 6 “Pulmotension” initiative, the prospective, longitudinal European Magnetic Resonance Imaging Study in PAH (EURO-MR) study was conducted in 4 European centres: Scottish Pulmonary Vascular Unit, Glasgow, United Kingdom; Medical University Graz, Graz, Austria; Sapienza University of Rome, Rome, Italy; and Department of Pulmonary Medicine, VU University Medical Center, Amsterdam, The Netherlands.

Between June 2003 and May 2010, patients with suspected PH were referred to their local PH centre for evaluation. Patients underwent clinical assessment, including New York Heart Association (NYHA) FC, RHC, 6MWD, and CMR, as described in Chapter 2.2

PH was diagnosed and classified according to current guidelines² and, if appropriate, disease-targeted therapy was initiated⁴. The initial therapy used was left to the discretion of the local PH physician. The 12-month follow-up evaluation assessment included 6MWD and CMR. Patients from the Glasgow centre also had a planned assessment (6MWD and CMR) after 4 months of treatment.

Patients in World Health Organization (WHO) group 1, WHO group 3, or WHO group 4, if ineligible for surgery, were included in the study.

Patients excluded from the analysis included:

- those unable to attend or who declined follow-up evaluation
- those who were not commenced on disease-targeted therapy after initial evaluation
- those with no evidence of PH at RHC
- those with PH associated with left heart disease (WHO group 2)
- those previously treated with disease-targeted therapy.

4.2.2 CMR Assessment

CMR was performed locally using a 1.5-T MRI scanner (Sonata Magnetom, Siemens; Erlangen, Germany) using the protocol described in Chapter 2.2.3. Individual scans from Graz, Rome and Amsterdam were coded by number and transferred to Glasgow for analysis. All scans were read in a blinded fashion by one of three observers (Dr Kevin Blyth, Dr Lindsey McLure, Dr Stephen Crawley) on a single MR work station using Argus analysis software.

Reproducibility and reliability of the CMR analysis were assessed by calculating the interobserver and intraobserver variability (presented as the mean bias and the 95% limits of agreement between two estimations) for the LV and RV measurements. In these retrospective calculations, 24 and 10 Glasgow CMR examinations were randomly selected to determine interobserver variability and intraobserver variability, respectively. For interobserver variability, CMR images were analysed by one observer (K.G.B or L.McL) and then reanalysed by another observer (S.F.C) who was blinded to the initial results. For intraobserver variability, scans were reanalyzed by the same observer, blinded to the initial results.

4.2.3 Statistical Analysis

For all variables, a normal distribution was verified using histograms and Kolmogorov–Smirnov tests. For demographic, haemodynamic, and CMR variables, mean \pm SD were calculated. For the entire cohort, comparison between baseline and 12-month follow-up was performed using the paired *t* test. A repeated measure 1-way ANOVA was used to compare the mean values in the smaller Glasgow subgroup. Variability analyses were compared using Bland–Altman analysis and *t* tests. The correlation between the change in CMRI variables and 6MWD as determined by Pearson correlation was also conducted. A *p* value < 0.05 was defined as statistically significant.

All calculations were performed using GRAPHPAD Prism (Version 5.00; GraphPad Software Inc; La Jolla, CA).

4.3 Results

4.3.1 Clinical characteristics

91 patients underwent assessment at baseline and after 12 months of disease-targeted therapy (Glasgow: 35; Amsterdam: 26; Rome: 17; and Graz: 13). Baseline characteristics for the entire cohort and by study centre are summarized in Table 4.2. The majority of patients were in WHO group 1, with idiopathic PAH and CTD-PAH the most common types. There were twice as many female patients as male patients, and the mean age at diagnosis was fifty-two years. Patients from the Glasgow subgroup had the poorest NYHA functional class and lowest 6MWD, with patients from the Rome subgroup having the best FC and 6MWD.

Table 4.2 Clinical characteristics for the study cohort

	Total Cohort (n=91)	Glasgow (n=35)	Amsterdam (n=26)	Rome (n=17)	Graz (n=13)
Age, yrs	52 ± 16	53 ± 16	46 ± 15	51 ± 15	61 ± 12
♀ : ♂	60 : 31	23 : 12	21 : 5	11 : 6	5 : 8
Body surface area, m ²	1.82 ± 0.23	1.80 ± 0.24	1.86 ± 0.25	1.78 ± 0.19	1.82 ± 0.22
Causes of PH, n (%)					
<i>WHO group 1</i>					
IPAH	42 (46.2)	16 (45.7)	10 (38.5)	12 (70.6)	4 (30.8)
PAH-CTD	15 (16.5)	5 (14.3)	7 (26.9)	1 (5.9)	2 (15.4)
PAH-CHD	4 (4.4)	1 (2.9)	2 (7.7)	0	1 (7.7)
Porto- pulmonary	4 (4.4)	2 (5.7)	0	0	2 (15.4)
Other ‡	6 (6.6)	0	4 (15.4)	1 (5.9)	1 (7.7)
<i>WHO group 3</i>					
PH because of hypoxic lung disease	5 (5.5)	3 (8.6)	0	1 (5.9)	1 (7.7)
<i>WHO group 4</i>					
CTEPH	15 (16.5)	8 (22.9)	3 (11.5)	2 (11.8)	2 (15.4)
NYHA functional class, n (%)					
I	2 (2.2)	0	0	0	2 (15.4)
II	37 (40.7)	11 (31.4)	12 (46.2)	11 (64.7)	3 (23.1)
III	48 (52.7)	23 (65.7)	13 (50.0)	6 (35.9)	6 (46.2)
IV	4 (4.4)	1 (2.9)	1 (3.8)	0	2 (15.4)
Baseline 6MWD, m	381 ± 127	316 ± 89	441 ± 140	461 ± 105	362 ± 126

6MWD indicates 6-minute walk distance; CTEPH, chronic thromboembolic pulmonary hypertension; IPAH, idiopathic PAH; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PAH-CHD, PAH associated with congenital heart disease; PAH-CTD, PAH associated with connective tissue disease; PAP, pulmonary artery pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; and WHO, World Health Organization

‡ PAH associated with HIV infection (n=1), heritable PAH (n=3), and drug and toxin-induced PAH (n=2).

All data expressed as mean \pm SD.

Baseline characteristics for the Group 1 PAH subgroup (n = 71) were:

Age (49 ± 15 yrs), 52 Female and 19 Male, Body Surface Area (1.80 ± 0.24 m²).

Within this subgroup most patients were in NYHA functional class II or III

(Fc I = 2pts; Fc II = 26pts; Fc III = 41pts; Fc IV = 2pts)

4.3.2 Pulmonary Haemodynamics

Baseline right heart catheterisation data for the study group is displayed in Table 4.3. These demonstrate significant pulmonary hypertension at the onset of disease-targeted therapy, with elevated mPAP and PVR and reduced cardiac index. Patients from the Glasgow subgroup had the most severe pulmonary haemodynamics, with those from the Graz subgroup having the least severe.

Table 4.3 Baseline pulmonary haemodynamics for the study cohort

Baseline haemodynamics, mean \pm SD	Total cohort (n = 91)	Glasgow (n = 35)	Amsterdam (n = 26)	Rome (n = 17)	Graz (n = 13)
Mean PAP, mmHg	46 \pm 15	47 \pm 14	49 \pm 16	44 \pm 16	38 \pm 10
PVR, Wood Units	10.0 \pm 6.3	11.2 \pm 5.3	10.6 \pm 8.2	9.4 \pm 5.9	6.3 \pm 3.4
Cardiac Index, L/min/m ²	2.4 \pm 0.9	2.1 \pm 0.5	2.7 \pm 1.0	2.3 \pm 0.6	2.9 \pm 1.2

Pulmonary haemodynamics for the PAH subgroup (n = 71) were mean PAP (46 \pm 15 mmHg), PVR (10.0 \pm 6.7 Wood Units) and Cardiac Index (2.5 \pm 0.9 L/min/m²).

4.3.3 Pulmonary Arterial Hypertension Therapy and Clinical Outcome

Medical therapy initiated at baseline within each centre is displayed in Table 4.4. Endothelin receptor antagonist monotherapy was the most frequently used treatment, with phosphodiesterase type-5 inhibitor the next most commonly used therapy. Upfront combination therapy was not used in the Glasgow centre, but was used in three patients in each of the other three centres.

Table 4.4 Initial PAH therapy for study patients

Initial medication, n (%)	Total (n = 91)	Glasgow (n = 35)	Amsterdam (n = 26)	Rome (n = 17)	Graz (n = 13)
Endothelin receptor antagonist	43 (47.3)	16 (45.7)	17 (65.4)	7 (41.2)	3 (23.1)
Phosphodiesterase type-5 inhibitor	23 (25.3)	16 (45.7)	1 (3.8)	4 (23.5)	2 (15.4)
Combination therapy	9 (9.9)	0	3 (11.5)	3 (17.6)	3 (23.1)
Calcium channel blocker	5 (5.5)	1 (2.9)	0	3 (17.6)	1 (7.7)
Prostanoid	5 (5.5)	1 (2.9)	3 (11.5)	0	1 (7.7)
Unknown	6 (6.6)	1 (2.9)	2 (7.7)	0	3 (23.1)

Within the Group 1 PAH subgroup (n = 71), the initial treatment was endothelin receptor antagonist (34 pts), phosphodiesterase type-5 inhibitor (16 pts), combination therapy (8 pts), calcium channel blocker (4 pts), prostanoid (5 pts) and unknown in four patients.

There was a significant increase in 6MWD after 12 months of disease-targeted therapy (381 ± 127 m at baseline, 443 ± 124 m at month 12; $p < 0.0001$).

At the 12-month follow-up, 5, 46, 36, and 4 patients were in NYHA FC I, II, III, and IV, respectively. Functional class had improved in 21 patients, was unchanged in 63 patients, and deteriorated in 7 patients.

4.3.4 CMR Assessment

CMR characteristics at baseline and after 12 months of disease-targeted therapy in the whole group (n=91) are displayed in Table 4.5. As expected, at baseline there was right ventricular dilatation (increased RVEDVI) and hypertrophy (increased RV mass index and VMI), impaired right ventricular systolic function with low stroke volume index and poor filling of the left ventricle (low LVEDVI). Left ventricular systolic function was normal.

Table 4.5. CMR Results for the whole study cohort

CMR Variable	Baseline (n = 91)	12 months (n = 91)	Mean Change	P Value
Right ventricle				
EDV index, ml/m ²	84 ± 26	86 ± 29	3 ± 19	0.22
Stroke volume index, ml/m ²	31 ± 8	36 ± 9	5 ± 11	<0.0001
Cardiac index, L/min/m ²	2.29 ± 0.63	2.62 ± 0.66	0.33 ± 0.72	<0.0001
Ejection fraction, %	41 ± 16	45 ± 15	5 ± 11	<0.0001
Mass index, g/m ²	52 ± 21	52 ± 19	0.4 ± 10.1	0.68
Left ventricle				
EDV index, ml/m ²	48 ± 13	53 ± 14	4 ± 12	0.0015
Stroke volume index, ml/m ²	32 ± 10	37 ± 10	4 ± 11	0.0002
Cardiac index, L/min/m ²	2.36 ± 0.63	2.66 ± 0.67	0.30 ± 0.68	<0.0001
Ejection fraction, %	67 ± 12	70 ± 10	4 ± 10	0.0007
Mass index, g/m ²	50 ± 12	52 ± 13	1 ± 7	0.11
Ventricular mass index	1.04 ± 0.37	1.03 ± 0.36	-0.01 ± 0.2	0.97

All data expressed as mean ± SD. EDV, end-diastolic volume.

After 12 months of therapy, there were significant increases in right ventricular function, whether measured by RV ejection fraction ($40.5 \pm 16.0\%$ at baseline, $45.2 \pm 14.7\%$ at month 12; $p < 0.0001$) or RV stroke volume index ($31.0 \pm 8.4 \text{ ml/m}^2$ at baseline, $35.9 \pm 9.3 \text{ ml/m}^2$ at month 12; $p < 0.0001$). Significant improvements were also noted in LVEDV index ($48.4 \pm 12.9 \text{ ml/m}^2$ at baseline, $52.5 \pm 14.1 \text{ ml/m}^2$ at month 12; $p = 0.0015$) and LV ejection fraction ($66.7 \pm 11.7\%$ at baseline, $70.3 \pm 9.8\%$ at month 12; $P = 0.0007$). Overall, there was no improvement or deterioration in the degree of RV dilatation (RVEDV index) or RV hypertrophy (ventricular mass index).

4.3.4.1 CMR Assessment in Group 1 Pulmonary Arterial Hypertension

CMR characteristics at baseline and after 12 months of disease-targeted therapy in the WHO Group 1 PAH group (n=71) are displayed in Table 4.6. Improvements in RV ejection fraction and stroke volume index, LV end-diastolic volume index and LV ejection fraction were again observed.

Table 4.6. CMR Results in Group 1 PAH Only

CMR Variable	Baseline (n=71)	12 months (n=71)	Mean Change	P Value
Right ventricle				
EDV index, ml/m ²	83 ± 27	84 ± 27	1 ± 17	0.58
Stroke volume index, ml/m ²	31 ± 9	36 ± 9	4 ± 10	0.0006
Cardiac index, L/min/m ²	2.35 ± 0.65	2.63 ± 0.65	0.29 ± 0.68	0.0008
Ejection fraction, %	41 ± 16	46 ± 14	5 ± 13	0.0016
Mass index, g/m ²	51 ± 21	51 ± 20	-0.2 ± 9	0.82
Left ventricle				
EDV index, ml/m ²	48 ± 13	52 ± 15	4 ± 12	0.0034
Stroke volume index, ml/m ²	32 ± 10	37 ± 11	4 ± 11	0.0011
Cardiac index, L/min/m ²	2.39 ± 0.63	2.68 ± 0.68	0.29 ± 0.66	0.0004
Ejection fraction, %	68 ± 11	71 ± 10	3 ± 9	0.0037
Mass index, g/m ²	50 ± 13	51 ± 13	1 ± 6	0.17
Ventricular mass index	1.05 ± 0.38	1.03 ± 0.38	-0.02 ± 0.2	0.85

All data expressed as mean ± SD. EDV, end-diastolic volume.

4.3.4.2 CMR Assessment by PH Treatment

Patients were stratified by initial PAH therapy, with CMR characteristics at baseline and after 12 months of endothelin receptor antagonist (Table 4.7) or phosphodiesterase type-5 inhibitor (Table 4.8) therapy are displayed below.

Table 4.7. CMR Results for patients receiving Endothelin Receptor Antagonist as initial therapy

CMR Variable	Baseline (n = 43)	12 months (n = 43)	Mean Change	P Value
Right ventricle				
EDV index, ml/m ²	80 ± 26	83 ± 29	2.7 ± 18.9	0.36
Stroke volume index, ml/m ²	32 ± 7	37 ± 10	5.4 ± 9.8	0.001
Cardiac index, L/min/m ²	2.3 ± 0.6	2.7 ± 0.7	0.4 ± 0.7	0.0018
Ejection fraction, %	43 ± 15	48 ± 14	4.9 ± 10.2	0.0035
Mass index, g/m ²	50 ± 25	49 ± 21	-1.1 ± 11.6	0.53
Left ventricle				
EDV index, mL/m ²	48 ± 12	53 ± 15	4.7 ± 10.2	0.0043
Stroke volume index, mL/m ²	32 ± 10	37 ± 10	4.7 ± 8.6	0.0002
Cardiac index, L/min/m ²	2.3 ± 0.5	2.7 ± 0.6	0.34 ± 0.53	0.0001
Ejection fraction, %	68 ± 9	72 ± 8	3.4 ± 7.9	0.0067
Mass index, g/m ²	50 ± 11	51 ± 12	0.9 ± 6.5	0.73

All data expressed as mean ± SD. EDV, end-diastolic volume.

Table 4.8. CMR Results for patients receiving Phosphodiesterase type-5 Inhibitor as initial therapy

CMR Variable	Baseline (n=23)	12 months (n=23)	Mean Change	P Value
Right ventricle				
EDV index, ml/m ²	89 ± 28	96 ± 31	6.4 ± 16.7	0.08
Stroke volume index, ml/m ²	27 ± 9	35 ± 5	7.1 ± 8.8	0.0008
Cardiac index, L/min/m ²	2.1 ± 0.6	2.6 ± 0.6	0.5 ± 0.6	0.0007
Ejection fraction, %	35 ± 17	41 ± 16	6.2 ± 10.1	0.0077
Mass index, g/m ²	55 ± 19	58 ± 21	3.4 ± 9.4	0.10
Left ventricle				
EDV index, ml/m ²	45 ± 12	52 ± 9	7.0 ± 9.4	0.0017
Stroke volume index, ml/m ²	28 ± 11	36 ± 8	7.8 ± 9.0	0.0004
Cardiac index, L/min/m ²	2.1 ± 0.7	2.7 ± 0.7	0.6 ± 0.7	0.0008
Ejection fraction, %	61 ± 15	69 ± 11	7.7 ± 12.6	0.0079
Mass index, g/m ²	48 ± 8	51 ± 10	3.3 ± 8.5	0.08

All data expressed as mean ± SD. EDV, end-diastolic volume.

Treatment with endothelin receptor antagonist therapy and phosphodiesterase type-5 therapy were both associated with improvements in right ventricular ejection fraction and stroke volume index, as well as improvements in left ventricular end-diastolic volume and ejection fraction. Neither therapy was associated with changes in the extent of right ventricular dilatation and hypertrophy. Direct comparison of the effectiveness of the two treatments was not performed.

4.3.4.3 CMR Assessment in the Glasgow subgroup

An additional clinical and CMR assessment was performed after 4 months of disease-targeted therapy in 27 of the 35 patients from Glasgow. CMR data for these patients are presented in Table 4.9

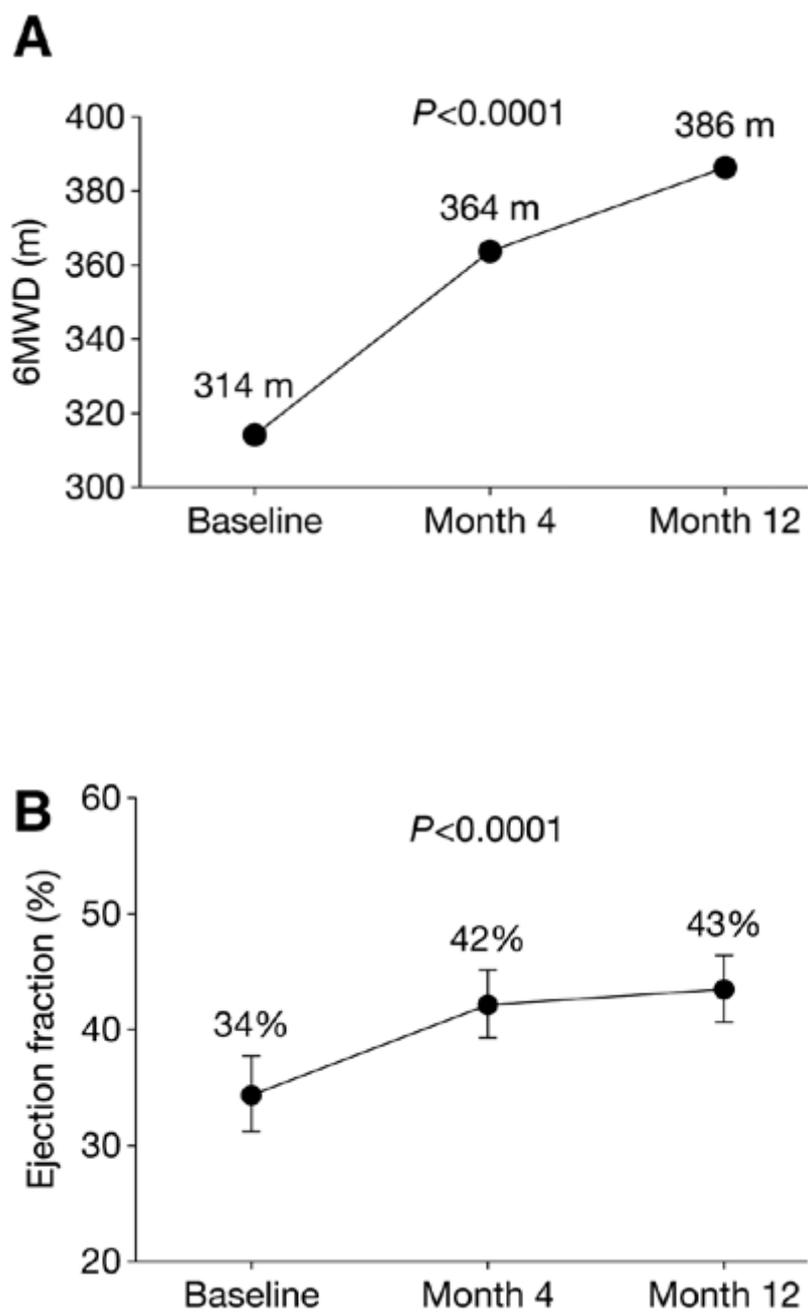
Table 4.9. CMR Results at baseline, 4 and 12 months for the Glasgow subgroup

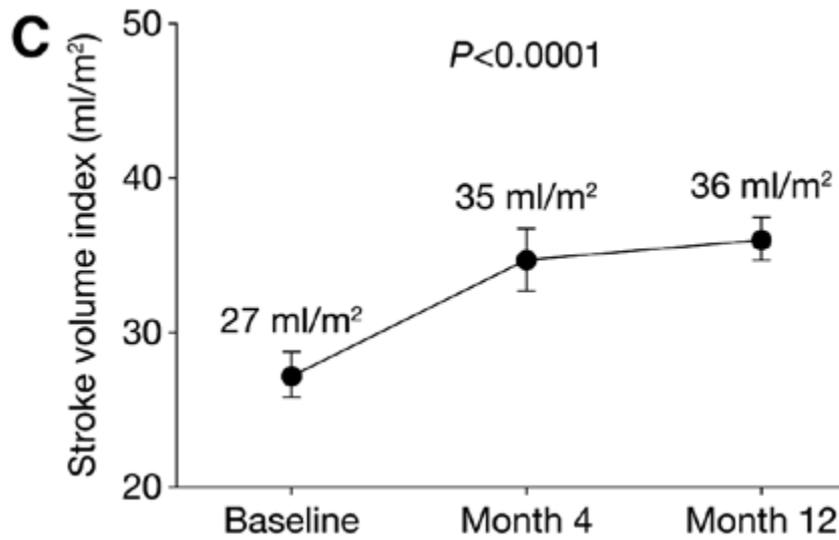
	Baseline	4/12	Change (0 to 4/12)	12/12	Change (0 to 12/12)	P value
Right Ventricle						
EDV Index, ml/m ²	88 ± 24	88± 24	-1 ± 13	90 ± 27	2 ± 19	0.61
SV Index, ml/m ²	27 ± 8	35± 11	7 ± 9	36 ± 8	9 ± 9	<0.0001
Cardiac Index	2.00 ± 0.41	2.43 ± 0.7	0.43 ± 0.67	2.58 ± 0.51	0.59 ± 0.68	0.0002
Ejection Fraction, (%)	34 ± 17	42± 15	8 ± 9	43 ± 15	9 ± 11	<0.0001
Mass Index, g/m ²	60 ± 22	57± 22	-3 ± 8	58 ± 21	-2 ± 13	0.28
Left Ventricle						
EDV Index, ml/m ²	45 ± 11	54± 15	8 ± 11	53 ± 13	8 ± 10	<0.0001
SV Index, ml/m ²	28 ± 9	37± 10	9 ± 8	37 ± 10	9 ± 9	<0.0001
Cardiac Index	2.04 ± 0.48	2.58 ± 0.68	0.54 ± 0.61	2.62 ± 0.59	0.58 ± 0.64	<0.0001
Ejection Fraction (%)	61 ± 13	69 ± 7	7 ± 11	69 ± 11	8 ± 11	0.0004
Mass Index, g/m ²	52 ± 9	53 ± 6	1 ± 6	54 ± 11	2 ± 8	0.47
VMI	1.2 ± 0.4	1.1 ± 0.4	-0.1 ± 0.2	1.1 ± 0.4	-0.1 ± 0.3	0.16

All data are expressed as mean ± SD. P-value refers to comparison from 1-way ANOVA. CMR indicates cardiac MRI; EDV, end-diastolic volume; SV, stroke volume; VMI, ventricular mass index

Within this subgroup, 6MWD increased throughout the study, and there were early improvements in RV ejection fraction and stroke volume index after four months of treatment, that were preserved at 12 months (Figure 4.1 A-C). There was no overall improvement or deterioration in RV hypertrophy or RV dilatation.

Figure 4.1 Changes in 6MWD, RV ejection fraction and RV stroke volume index after four and twelve months of disease-targeted PAH therapy



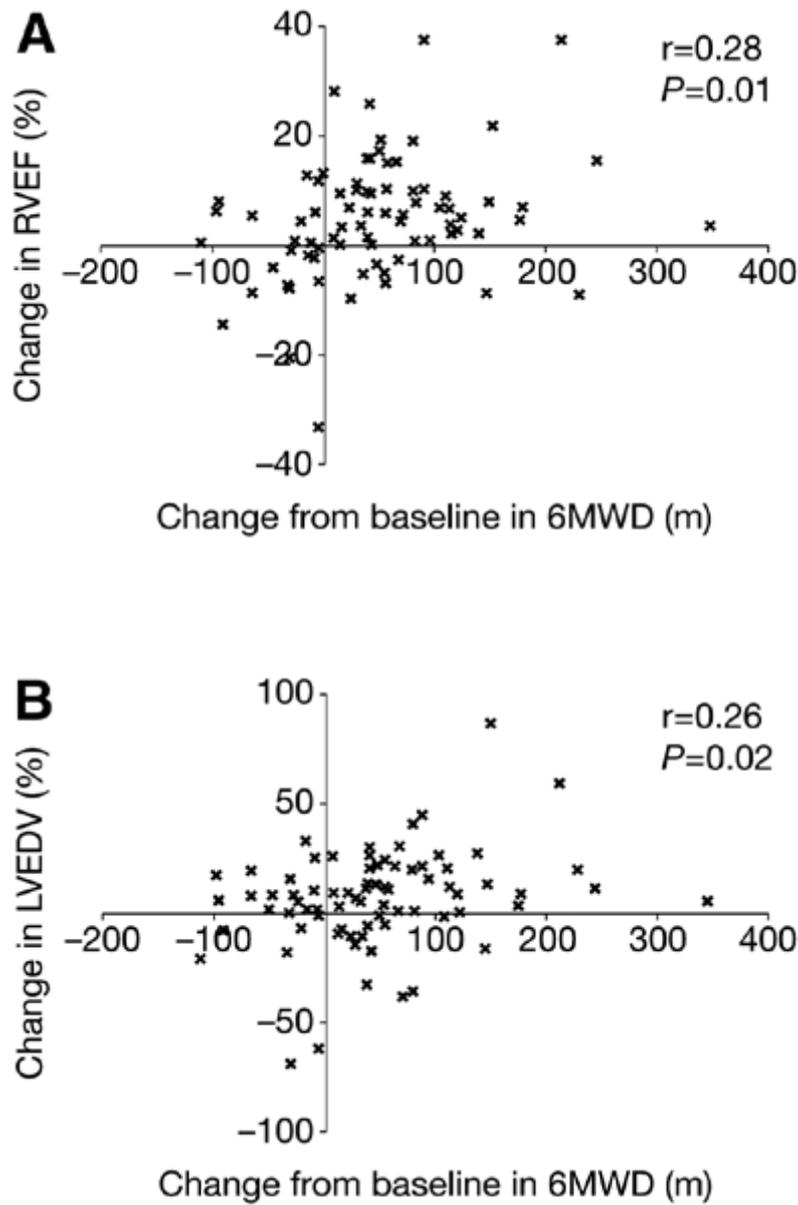


Change in 6-minute walk distance (6MWD; **A**); right ventricular (RV) ejection fraction (**B**); and RV stroke volume index (**C**) after 4 months and 12 months of disease-targeted therapy in the Glasgow cohort. Improvements occurred after 4 months of treatment and were preserved at 12 months.

4.3.4.4 Association of CMR Variables With 6MWD

Significant correlations were observed between change in 6MWD and change in RV ejection fraction ($r = 0.28$, $p = 0.01$), and between change in 6MWD and % change in LV end-diastolic volume ($r = 0.26$, $p = 0.02$), although correlation coefficients were low (Figure 4.2). There was no significant correlation between change in 6MWD and changes in right ventricular stroke volume ($p = 0.054$) or cardiac output ($p = 0.08$).

Figure 4.2 Correlation between changes in CMR variables and $\Delta 6\text{MWD}$



Pearson correlation between the absolute change in right ventricular ejection fraction (RVEF; **A**); and the % change in left ventricular end-diastolic volume (LVEDV; **B**) with change in 6MWD, for the whole study cohort.

4.3.4.5 Interobserver and Intraobserver variability of CMR variables

Analysis of interobserver variability in a subgroup of 24 patients from the Glasgow centre demonstrated lower interobserver variability for left ventricular measurements than for those in the right ventricle. (Table 4.10)

Table 4.10 Inter-observer variability of CMR measurements

	Mean Bias	95% Limits of Agreement
LV ejection fraction (%)	-1.7	-16.2 to 12.3
LV end-diastolic volume (ml)	1.2	-17.4 to 19.7
LV stroke volume (ml)	-0.3	-14.3 to 13.6
LV mass (g)	-8.2	-42.8 to 26.5
RV ejection fraction (%)	0.8	-9.4 to 11.0
RV end-diastolic volume (ml)	-11.6	-45.0 to 22.0
RV stroke volume (ml)	-4.2	-15.0 to 6.6
RV mass (g)	13.7	-14.0 to 41.3

Note that measures of end-diastolic volume, stroke volume and mass are not indexed for body surface index. LV, left ventricular; RV, right ventricular.

Intra-observer variability data was assessed in 10 patients at the Glasgow centre selected at random. Scans were reanalysed blinded to the initial results. Intra-observer variability was low for volume measurements (EDV, stroke volume, and EF) but higher for mass measurements. (Table 4.11)

Table 4.11 Intra-observer variability of CMR measurements

	Mean Bias	95% Limits of Agreement
LV ejection fraction (%)	-1.2	-6.5 to 4.2
LV end-diastolic volume (ml)	-0.2	-3.6 to 3.3
LV stroke volume (ml)	-1.3	-6.5 to 3.8
LV mass (g)	-5.4	-15.0 to 4.1
RV ejection fraction (%)	-0.8	-6.7 to 5.0
RV end-diastolic volume (ml)	-0.1	-12.2 to 12.4
RV stroke volume (ml)	-1.2	-7.0 to 4.5
RV mass (g)	6.7	-13.3 to 26.6

Note that measures of end-diastolic volume, stroke volume and mass are not indexed for body surface index. LV, left ventricular; RV, right ventricular.

4.4 Discussion

The current study prospectively assessed the use of CMR before and during PAH disease-targeted therapy. It has demonstrated that both left-sided and right-sided variables should be included when assessing cardiac function in patients with precapillary PH. Stroke volume index, cardiac index, and ejection fraction were associated with response when measured in either the left or the right heart, and LVEDV index was also associated with therapeutic response. In the past, it has been suggested that the initial benefits achieved with some disease-targeted therapies are not maintained during long-term treatment³⁰¹; however, the data from the Glasgow subgroup demonstrates that the improvement in stroke volume index, cardiac index, and ejection fraction at four months was still present at twelve months.

The results of the current study support the data from prior trials studying changes in CMR-derived RV variables during treatment for PH. There has been only one major clinical trial of PAH therapy that has used right ventricular function as a pre-specified primary or secondary endpoint. In the Sildenafil versus endothelin receptor antagonist for pulmonary hypertension (SERAPH) study, 26 patients with IPAH or CTD-PAH and WHO Fc III, were randomized to sildenafil (50 mg twice daily for 4 weeks, then 50 mg three times daily) or bosentan (62.5 mg twice daily for 4 weeks, then 125 mg twice daily) over 16 weeks. Patients on sildenafil who completed the protocol revealed a significant reduction in RV mass (as measured by CMR) ($p = 0.015$) compared with baseline, whereas there was no reduction observed in the Bosentan treated patients²⁹³. Retrospective review of twelve patients from the Ambrisentan in Pulmonary Arterial Hypertension: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Multicenter Efficacy Study (ARIES-1) clinical trial and extension phase reported that two years of ambrisentan therapy resulted in an increased right ventricular ejection fraction at CMR, but no improvement in RV end-diastolic volume index or RV mass³⁰².

Some single-centre studies have prospectively examined the effect of PAH therapy on right ventricular structure and function. Twelve months of intravenous Epoprostenol was found to improve right ventricular stroke volume at CMR in a small cohort of eleven patients, although there was neither progression nor regression of RV hypertrophy and dilatation¹¹⁵. In fifteen patients with PAH already receiving bosentan, the addition of Sildenafil for three months resulted in right ventricular reverse remodelling, with a decrease in the RV

dilatation and hypertrophy that had progressed whilst on bosentan monotherapy³⁰³. Similarly, in twenty patients with PAH who were clinically worsening despite prostanoid therapy, the addition of Sildenafil resulted in a reduction in right ventricular end-diastolic diameter at echocardiography³⁰⁴.

Lee *et al* used non-invasive IGR haemodynamic measurements (stroke volume and pulmonary blood flow) to detect a treatment response in patients with precapillary PH receiving de novo or modified disease-targeted therapy, and found that these measurements may be more responsive to change than 6MWD in fitter patients³⁰⁵. Serial measurement of B-type natriuretic peptide (BNP) was performed in approximately 50% of the patients enrolled in the aforementioned ARIES-1 study of ambrisentan therapy²⁹⁶. In the placebo group BNP concentrations increased slightly from baseline, whereas active treatment was associated with a reduction in BNP in 30% of patients in the 5mg/day group and 45% of patients in the 10mg/day group. NT-ProBNP measurement was performed in 136 of the 185 patients in the EARLY study of bosentan therapy in NYHA FC II patients, with a reduction in NT-ProBNP observed in the bosentan group compared with the placebo group (mean treatment effect -471 ng/L, $p = 0.0003$) that corresponded to the improvements observed in pulmonary haemodynamics⁷⁹.

The prognostic significance of changes in RV function after PAH therapy have also been investigated. In sixteen patients with PAH evaluated at baseline and after 12 months of bosentan, treatment resulted in improvements in cardiac index, pulmonary vascular resistance, and 6MWD, with a trend toward improvement in RV stroke volume (70 +/- 27 to 81 +/- 30 ml; $p = 0.08$), but no change in RV ejection fraction (RVEF) or RV end-diastolic volume. However, 6MWD improved more in patients in whom RVEF increased compared with those with stable or decreased RVEF (+98m vs -37m; $p = 0.01$)³⁰⁶. A large cohort of 76 newly diagnosed PAH patients were evaluated at baseline and after 12 months of therapy (34% endothelin antagonists, 13% PDE-5 inhibitor, 11% prostacyclin, 41% combination therapy)³⁰⁷. Overall, despite improvements in PVR and cardiac index, PAH therapy did not improve 6MWD (421m v 425m; $p = 0.73$), or CMR measurements of right ventricular structure and function. However, at survival analysis RVEF measured at baseline was a better predictor of mortality than PVR, and that changes in RVEF after 12 months predicted long-term outcome, whereas changes in PVR did not. In 25% of patients with a reduced PVR after treatment, there was deterioration in RV function and this was associated with a poor prognosis. Further analysis of the same cohort of patients was performed to identify the minimal important difference (MID) in stroke volume at CMR

that would constitute a clinically significant change. A 10ml change in stroke volume following PAH therapy was found to be clinically relevant, and changes in stroke volume correlated with changes in 6MWD¹¹⁹.

The multi-centre nature of the current study provided the opportunity to study the consistency of the CMR findings in several subanalyses. Although the number of patients in each centre was small and the analysis was not powered for comparison, at the majority of centres, there was a trend toward improvement or at least no deterioration in the CMR variables significantly associated with therapeutic response in the main analysis. Similarly, when stratified for initial PAH therapy the same variables were identified, and despite the small study size, statistical significance was achieved. These findings support the wider applicability of CMR for the follow-up patients with PH on disease-targeted therapy.

In this analysis, identification of patients suitable for therapy, and initial therapy used, was at the discretion of the responsible physician, although all units will have followed recognized national and international guidelines^{2, 236}. This suggests that the results obtained reflect real clinical practice, and that the findings are likely to be pertinent for other treatment centres, and may also provide insight into different treating practices. However, differences between treatments cannot be determined because the study was not designed to collect data on changes in therapy during the study duration. The consistency of the findings when stratified by baseline PAH-specific therapy suggests that although acting via different pathways, the net effect of these treatments on pulmonary vasculature and RV function may be similar.

Whilst it is well established that RV function is associated with outcome in PAH, some left heart variables are known to better correlate with overall cardiac function than RV function. LV end-diastolic volume is more closely related to stroke volume than RVEDV⁴², and data from echocardiography studies have demonstrated that diastolic eccentricity index, which include LV size, is closely associated with outcome in PAH⁷². Several groups have also demonstrated that poor LV output in PH is related to poor filling of the LV¹¹⁷. This underfilling of the LV, a consequence of poor filling on the left side because of prolonged RV contraction time⁴¹, contributes to decreased SV⁴². For this reason, it is likely that LVEDV may be a particularly important variable because it reflects both SV and increased RV contraction time, and highlights the importance of LV volume measurements in patients with PH.

There are several limitations of the current study. It is not possible to provide information on patients who underwent initial CMR but were then unable to attend for subsequent CMR imaging due to patient preference, geographical limitations, illness or death, and clearly this could lead to a ‘survivor’ bias within the results obtained. Similarly, the overall PH population from which the study cohort was drawn could not be determined in detail, so it is not possible to assess if there was any bias in study entry. In this study a heterogeneous population of patients with PH were enrolled, and, unlike previous studies, it was not restricted to patients with PAH. Although disease-specific therapy is only recommended in management guidelines for PAH², in clinical practice, these treatments are also often used in WHO groups 3, 4, and 5. The multi-centre nature of the study meant that it was not possible to obtain information on changes to PAH therapy that may have occurred during the study period. It would have been valuable to determine the relationship between the CMR variables and patient outcome, however outcome data were not collected in this study, and retrospective analysis was therefore not feasible. Another limitation of the current study is that inter-observer and intra-observer reproducibility were assessed via reanalysis of the same pre-derived images, rather than an alternative method such as ‘test-retest’, potentially leading to an overestimation of CMR reproducibility.

4.5 Conclusions

The study in this chapter has demonstrated that disease-targeted PAH therapy improves right ventricular function, as measured by CMR. Improvement in RV function occurs early, within 4 months of treatment, and is preserved at 12 months. On-treatment changes in CMR variables from the left and right sides of the heart reflect changes in functional class and exercise capacity, and the robust and non-invasive nature of CMR imaging means that it has potential to be a useful indicator of therapeutic response in future trials of PH therapy.

Chapter 5

Prognostic value of cardiac dysfunction in IPAH and CTD-PAH

5.1 Introduction

In Chapter 4 it was shown that direct measurement of right ventricular function with CMR could be used to assess the benefit of disease-targeted therapy in patients with PAH, including PAH due to connective tissue disease (CTD-PAH). PAH is a well recognized complication of connective tissue diseases, most commonly systemic sclerosis (SSc)²⁹⁹. Estimates of SSc-PAH prevalence range from 10% to 12% depending on the diagnostic modality used^{308, 309}. Following the development of angiotensin-converting enzyme therapy to treat renal crises, PAH is now one of the leading causes of morbidity and mortality in SSc³¹⁰. Therefore, patients with SSc typically undergo annual screening for pulmonary hypertension with transthoracic echocardiogram, and are referred for diagnostic RHC if they have an elevated TR jet velocity / PASP estimate or unexplained dyspnoea³¹¹. In the Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma (PHAROS) cohort study, 251 “at-risk” SSc patients were prospectively followed for 2.5 +/- 1.2 years (“at risk” = echo SPAP >40 mmHg, TLCO <55% predicted, or % FVC : %TLCO > 1.6)³¹². A low TLCO, high FVC:TLCO, and entry echo SPAP > 40 mmHg were strongly associated with future pulmonary hypertension at cardiac catheterisation, however of the 35 new PH patients, 22 had PAH, 10 had pulmonary venous hypertension, and 3 had pulmonary hypertension due to interstitial lung disease (WHO group 3). The symptoms associated with SSc-PAH are similar to those observed in IPAH (dyspnoea, fatigue, syncope, and chest pain), however patients may have minimal or no dyspnoea at diagnosis³¹³, and can instead attribute their deteriorating functional status to their longstanding SSc. This can be further complicated by the high prevalence of coexisting musculoskeletal involvement³¹⁴, which can also limit the utility of the 6MWT and cardiopulmonary exercise test during diagnostic assessment³¹⁵. As discussed in Section 1.7.2.4, PAH therapies have been extensively studied in patients with connective tissue disease, usually as a subgroup analysis of clinical trials containing patients with PAH of all causes. Treatment effects on exercise capacity and survival tend to be more modest in CTD-PAH compared with IPAH^{171, 172}.

5.1.1 Pathogenesis of SSc-PAH

As in idiopathic PAH, the pathogenesis of SSc-PAH is characterized by progressive remodelling of small or medium sized pulmonary arteries. Within the endothelium there is an imbalance between antiproliferative mediators (e.g. prostacyclin and nitric oxide) and vasoactive proliferative mediators (endothelin-1 and thromboxane A₂), with resultant pulmonary artery vasoconstriction and cellular proliferation³¹¹. Subsequent ischaemic reperfusion injury within the pulmonary vasculature can promote further cytokine release, fibrosis and intraluminal microthrombosis³¹⁶. There is also evidence of a higher frequency of obstructive vascular lesions in pulmonary veins/preseptal venules in CTD-PAH than in IPAH³¹⁷. The presence of autoantibodies and autoimmune dysregulation are also important in SSc-PAH^{318, 319}. A recent study has suggested that endothelin-1 receptor type A and angiotensin II type-1 receptor antibodies are common in SSc, can contribute to pathological vascular remodelling, and are associated with increased mortality³²⁰. Antiendothelial cell autoantibodies and antifibroblast autoantibodies may also contribute to endothelial cell apoptosis, fibroblast proliferation and collagen synthesis in SSc-PAH^{321, 322}. In addition to pulmonary vascular abnormalities, SSc can also exert a primary inflammatory effect on the heart, with subsequent myocardial fibrosis and impaired microcirculatory function³²³.

5.1.2 PAH in Other Connective Tissue Diseases

Whilst pulmonary arterial hypertension occurring in the setting of an underlying connective-tissue disease (CTD-PAH) is most frequently observed in patients with systemic sclerosis (SSc-PAH), it can also be found in mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE), dermatomyositis/polymyositis (DM/PM), rheumatoid arthritis (RA), undifferentiated connective tissue disease (UCTD) and Sjogren's syndrome. In a recent registry of all incident cases of CTD-PAH diagnosed in the UK between 2001 and 2006, 74% of patients had underlying systemic sclerosis²⁹⁹. Compared with SSc-PAH, higher survival rates were observed in PAH associated with SLE, and associated with DM/PM. These findings have been supported by data from the REVEAL registry which have demonstrated that systemic sclerosis accounts for 62% (399 out of 641) of CTD-PAH patients, and that SSc-PAH is a unique phenotype with the highest BNP levels, lowest DLCO, and poorest survival of all CTD-PAH subgroups¹⁷³. Survival in PAH associated with RA is felt to be comparable to survival in IPAH³²⁴.

5.1.3 Survival differences between IPAH and CTD-PAH

It is well recognized that despite similar baseline demographics and often less severe pulmonary haemodynamics, survival is worse in CTD-PAH, particularly SSc-PAH, than in IPAH^{165, 173, 233, 234}. The recent UK registries of incident, treatment-naïve cases of CTD-PAH and IPAH has demonstrated one-, two-, and three-year survival rates of 78%, 58%, and 47% in SSc-PAH²⁹⁹, compared with 93%, 84%, and 73% in IPAH¹⁸⁵. At baseline right heart catheterisation, patients with IPAH had higher mPAP (54 mmHg v 42 mmHg), higher PVR (12.8 Wood Units v 8.9 Wood Units) and lower cardiac index (2.1 L/min/m² v 2.43 L/min/m²) than those with SSc-PAH. There was no difference in WHO functional class distribution between the groups, however the SSc-PAH cohort were older (64 yrs v 50yrs), more female (82% v 70%), and had poorer baseline 6MWD (231m v 292m) than the IPAH cohort.

Similar findings have been observed in the REVEAL registry of new and previously diagnosed PAH patients, which included 1251 patients with IPAH and 641 patients with CTD-PAH, of which 399 had SSc-PAH. One year survival rates were significantly lower in CTD-PAH than in IPAH (86% v 93%, $p < 0.0001$), and lower still in the SSc-PAH subgroup (82% at 1 year)¹⁷³. Haemodynamics at the time of diagnostic RHC were less severe in CTD-PAH (mPAP 45 mmHg, PVR 9.8 WU, Cardiac Index 2.5 L/min/m²) than in IPAH (mPAP 53 mmHg, PVR 12.5 WU, Cardiac Index 2.3 L/min/m²). As before, WHO functional class distribution was the same in both groups, however the CTD-PAH cohort were older (57 yrs v 50yrs), more female (90% v 79%), and had poorer baseline 6MWD (301m v 329m) and higher NT-ProBNP (2313 v 1263 pg/ml) than the IPAH cohort. It should be noted that the time interval between diagnostic RHC and enrolment was 41.1 ± 44.1 months in the IPAH group and 27.2 ± 29.9 months in the CTD-PAH group.

Therefore, the reason for the poorer survival observed in CTD-PAH, particularly SSc-PAH, remains unclear. In the UK registry described earlier, Condliffe *et al* found that age, sex, mixed venous oxygen saturation, and WHO functional class were all independent predictors of survival in isolated SSc-PAH²⁹⁹. Similarly, in the REVEAL registry patients with SSc-PAH who were at a particularly high risk of death included elderly men and those with low baseline systolic blood pressure or 6MWD, or markedly elevated RAP or PVR³²⁵. Lefevre *et al* further investigated the prognostic factors for survival in SSc-PAH via a meta-analysis of 22 studies including 2244 patients³²⁶. They reported that age, male

sex, DLCO, pericardial effusion, and many of the prognostic factors typically observed in IPAH (including 6MWD, right atrial pressure, and cardiac index) were also prognostic factors in SSc-PAH.

The importance of left ventricular dysfunction in determining survival in the two conditions has been investigated less frequently. In their 2006 study, Fisher *et al* reported that although patients with SSc-PAH had a higher prevalence of LV morphologic and functional abnormalities than was seen in IPAH, the presence of left heart disease was not associated with increased mortality²³³. However, in the recent meta-analyses and registry-based studies investigating predictors of survival in CTD-PAH and SSc-PAH, the role of left ventricular dysfunction, particularly diastolic dysfunction, has not been explicitly explored^{325, 326}. The experiment in this chapter tested the hypothesis that right and left sided cardiac dysfunction could influence prognosis in CTD-PAH, and explain the survival difference between idiopathic and connective-tissue disease associated PAH.

5.2 Methods

5.2.1 Patients

Patients with a new suspected diagnosis of pulmonary hypertension were admitted for diagnostic assessment to the Scottish Pulmonary Vascular Unit in Glasgow, UK between December 2003 and July 2011. Patients underwent clinical assessment including New York Heart Association (NYHA) functional class (FC), right heart catheterisation (RHC), six-minute walk distance (6MWD) and CMR. CMR imaging was performed within 72 hours of RHC. At the time of assessment, no patient had received any specific pulmonary hypertension treatment (other than anticoagulation with warfarin).

Following RHC, PH was diagnosed and classified according to current guidelines and, if appropriate, disease-targeted therapy was initiated². IPAH was diagnosed when PAH was present (i.e. mPAP > 25mmHg, PAWP ≤ 15mmHg, PVR ≥ 3 mmHg/L/min) but no underlying cause for the PAH could be identified. CTD-PAH was diagnosed when PAH was present in the setting of connective-tissue disease, as agreed by the MDTM. The underlying connective tissue disease was categorized through review of clinical notes and MDT documentation. Patients with evidence of interstitial lung disease on computed tomography scan, as reported by Consultant Radiologist, and impaired spirometry (Forced Vital Capacity (FVC) <70% predicted) were excluded from the study. Survival was determined on 31st December 2011, using the computerized SCI-Store system if required. Date of diagnosis was taken as the date of the diagnostic right heart catheter.

5.2.2 CMR Image Acquisition

CMR image acquisition was performed as described in Methods Section 2.2.3

5.2.3 CMR Image Analysis

CMR image analysis was performed as described in Methods Sections 2.2.3.5 and 2.2.3.6.

5.2.4 Statistical analysis

For all variables, normal Gaussian distribution was verified using histograms and Kolmogorov-Smirnov tests. For demographic, CMR, and haemodynamic variables mean values \pm one standard deviation (\pm SD) were calculated. Student's t-test or Mann-Whitney test was used to compare values between the groups. Discrete data were compared using the Chi-squared test. Survival from date of diagnosis was assessed using the Cox regression and Kaplan–Meier methods, with comparisons performed by log-rank test. Correlations between normally distributed CMR and haemodynamic variables were tested by Pearson's method. Post-hoc analysis was carried out by stratifying patients into high and low RV function using the median baseline RVSVI, and into normal or abnormal LV systolic function using an LVEF threshold of 60%.

Statistical analysis was performed using Statview version 5.0.1 (SAS Institute, Cary, NC, USA) and GRAPHPAD Prism (Version 5.00; GraphPad Software Inc, La Jolla, CA, USA)

5.3 Results

5.3.1 Baseline Characteristics

Between December 2003 and July 2011 138 patients fulfilled the criteria for pulmonary arterial hypertension and underwent diagnostic assessment with CMR and right heart catheterisation. Of these 74 were diagnosed with IPAH and 38 were diagnosed with CTD-PAH. The remaining 26 had portopulmonary hypertension or PH due to congenital heart disease. In the CTD-PAH group the underlying connective-tissue disease was: systemic sclerosis (21 patients), rheumatoid arthritis (5 patients), systemic lupus erythematosus (4 patients), mixed connective tissue disease (2 patients), dermatomyositis (1 patient), unknown (5 patients).

Baseline demographics, clinical data and lung function are presented in Table 5.1. There was no significant difference in age, NYHA functional class, lung function or 6MWD between the two groups. There were a higher proportion of females in the CTD-PAH group.

Table 5.1 Baseline characteristics of the study groups

	IPAH (n = 74)	CTD-PAH (n = 38)	P Value
Age (years)	58 (\pm 17)	60 (\pm 14)	0.42
Sex (♀; ♂)	43 : 31	33 : 5	
NYHA Fc, no (%)			0.81 [†]
I / II	14 (18.9)	9 (23.7)	
III	53 (71.6)	25 (65.8)	
IV	7 (9.5)	4 (10.5)	
Body Surface Area (m ²)	1.77 (\pm 0.23)	1.68 (\pm 0.26)	0.07
Resting Heart Rate (bpm)	78 (\pm 14)	81 (\pm 17)	0.28
6MWD (m)	256 (\pm 132)	214 (\pm 128)	0.11
Echo TRPG (mmHg)	70 (\pm 18)	66 (\pm 28)	0.32
FEV ₁ (% predicted)	85 (\pm 17)	86 (\pm 23)	0.96
FVC (% predicted)	103 (\pm 19)	99 (\pm 26)	0.39
TLCO (% predicted)	43 (\pm 22)	41 (\pm 19)	0.79

IPAH, idiopathic pulmonary arterial hypertension; CTD-PAH, connective tissue disease associated pulmonary arterial hypertension; NYHA, New York Heart Association; bpm, beats per minute; 6MWD, six minute walk distance; TRPG, tricuspid regurgitation pressure gradient; FEV₁, forced expiratory volume in 1s; FVC, forced vital capacity; TLCO, transfer factor for the lung for carbon monoxide

Data presented as mean (\pm SD) otherwise specified

Student t-test or Mann-Whitney Rank Sum Test as appropriate

† - using the Chi-squared test for categorical variables

5.3.2 CMR Analysis

CMR results for the two groups are presented in Table 5.2. Both groups had significant right ventricular dilatation and RV hypertrophy, and poor RV systolic function. Both groups also had impaired left ventricular filling (reduced LVEDV) but well preserved left ventricular ejection fraction. Patients with IPAH had greater right ventricular hypertrophy than those with CTD-PAH (VMI 1.16 v 0.99, $p = 0.03$). Left atrial volume was lower in IPAH than CTD-PAH (23 vs 33 ml/m², $p < 0.0001$).

Table 5.2 Baseline CMR results for the study groups

	IPAH (n = 74)	CTD-PAH (n = 38)	P Value
Right Ventricle			
EDV Index (ml/m ²)	85 (± 24)	80 (± 21)	0.27
Stroke Volume Index (ml/m ²)	27 (± 9)	27 (± 8)	0.96
Ejection Fraction (%)	34 (± 13)	37 (± 17)	0.27
Mass (g/m ²)	57 (± 20)	49 (± 16)	0.04
Left Ventricle			
EDV Index (ml/m ²)	45 (± 14)	45 (± 14)	0.85
Stroke Volume Index (ml/m ²)	28 (± 9)	29 (± 10)	0.68
Ejection Fraction (%)	63 (± 13)	64 (± 13)	0.60
Mass (g/m ²)	50 (± 12)	52 (± 12)	0.38
Ventricular Mass Index	1.16 (± 0.36)	0.99 (± 0.33)	0.03
Left Atrial Volume (ml/m ²)	23 (± 11)	33 (± 11)	<0.0001

IPAH, idiopathic pulmonary arterial hypertension; CTD-PAH, connective tissue disease associated pulmonary arterial hypertension; EDV, end-diastolic volume

Data presented as mean (±SD) otherwise specified

Student t-test or Mann-Whitney Rank Sum Test as appropriate

5.3.3 Pulmonary Haemodynamics

Baseline haemodynamics for the study groups are presented in Table 5.3. In IPAH there was higher mPAP (50 v 43mmHg, $p = 0.01$) and higher PVR (12.3 v 10.3 Wood Units, $p = 0.07$) than in CTD-PAH. There was no difference in cardiac index, PAWP or right atrial pressure between the groups.

Table 5.3 Baseline haemodynamics for the study groups

	IPAH (n = 74)	CTD-PAH (n = 38)	P value
Mean PAP (mmHg)	50 (\pm 14)	43 (\pm 12)	0.01
PAWP (mmHg)	8 (\pm 4)	7 (\pm 5)	0.58
Cardiac Index (L/min/m ²)	2.1 (\pm 0.6)	2.3 (\pm 0.7)	0.14
PVR (Wood Units)	12.3 (\pm 5.4)	10.3 (\pm 5.4)	0.07
RAP (mmHg)	8 (\pm 6)	9 (\pm 10)	0.42

IPAH, idiopathic pulmonary arterial hypertension; CTD-PAH, connective tissue disease associated pulmonary arterial hypertension; PAP, pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary artery pressure; RAP, right atrial pressure.

Data presented as mean (\pm SD)

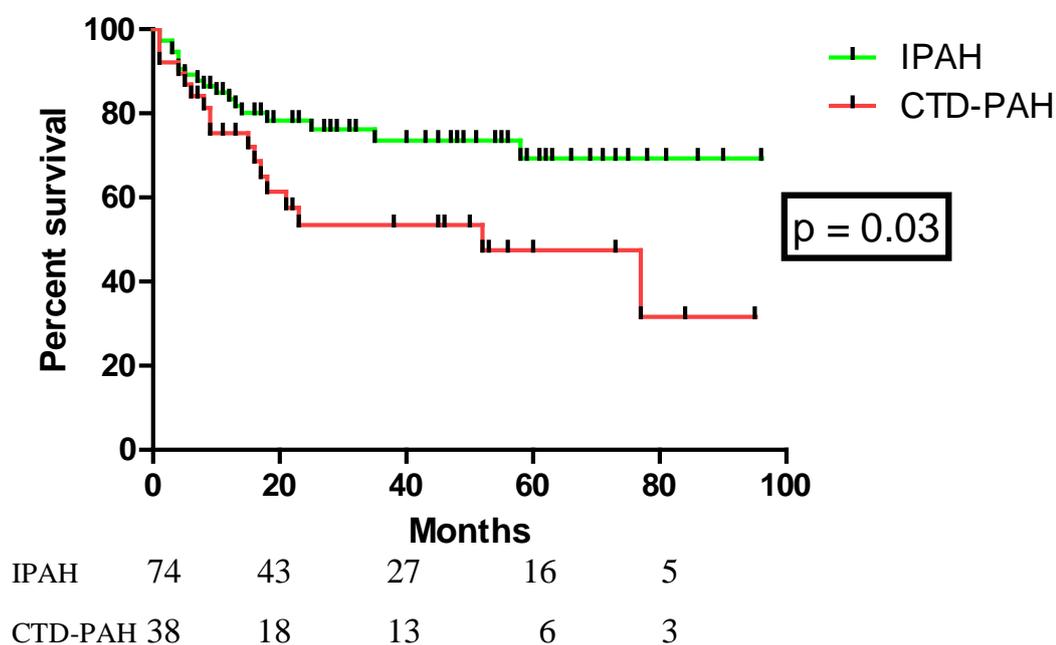
5.3.4 PAH Treatment

Initial therapy in both groups was in accordance with ERS guidelines and there was no difference in the distribution of initial therapies between the IPAH and CTD-PAH groups ($p = 0.38$, Chi-squared test). In IPAH this was Prostanoid (4 patients), Phosphodiesterase-5 Inhibitor (36 patients), Endothelin Receptor Antagonist (31 patients), Calcium Channel Blocker (3 patients). In CTD-PAH this was Prostanoid (1 patient), Phosphodiesterase-5 Inhibitor (13 patients), Endothelin Receptor Antagonist (22 patients), Other (2 patients). PDE-5 or ERA monotherapy was used in 67/74 (91%) of IPAH and 35/38 (92%) of CTD-PAH patients.

5.3.5 Survival Analysis

During a median follow-up period of 22 months (IQR: 9 to 53 months) 17 CTD-PAH and 18 IPAH patients died. Survival was greater in IPAH than in CTD-PAH (Log-Rank test – $p = 0.03$) (Figure 5.1). Survival rate in IPAH was 83% at 1yr, 76% at 2yrs and 74% at 3yrs, and in CTD-PAH was 75% at 1yr, 53% at 2yrs and 53% at 3yrs.

Figure 5.1 Kaplan Meier survival estimates of the study cohort stratified by diagnosis



Survival was better in IPAH than in CTD-PAH ($p = 0.03$).

IPAH, idiopathic pulmonary arterial hypertension; CTD-PAH, connective-tissue disease associated pulmonary arterial hypertension

5.3.5.1 Predictors of Survival at Univariate Analysis

Univariate analysis was performed on the entire cohort to identify clinical, haemodynamic and CMR predictors of survival and the results are presented in Table 5.4. Age, functional class, the presence of connective-tissue disease (vs idiopathic PAH), TLCO, PVR, Cardiac Index, RAP, stroke volume (RV and LV), right ventricular ejection fraction and left ventricular end-diastolic volume were all predictors of survival.

Table 5.4 Univariate Cox proportional hazards analysis

<u>Variable</u>	<u>Hazard Ratio</u>	<u>95% C.I.</u>	<u>P Value</u>
Age, yrs	1.0446	1.018 - 1.074	0.001
6MWD, (m)	0.991	0.988 - 0.994	<0.0001
Functional Class	3.637	1.877 - 7.048	0.0001
FEV1 (% pred)	1.002	0.983 - 1.021	0.86
FVC (% pred)	1.008	0.992 - 1.025	0.34
TLCO (%)	0.951	0.927 - 0.976	0.0001
Disease (CTD-PAH)	2.047	1.053 - 3.976	0.035
mPAP, mmHg	1.003	0.981 - 1.025	0.80
Cardiac Index, L/min/m ²	0.313	0.156 - 0.630	0.001
PVR, Wood Units	1.068	1.011 - 1.128	0.02
RAP, mmHg	1.097	1.047 - 1.149	<0.0001
RV EDVI, ml/m ²	1.000	0.985 - 1.014	0.97
RV SVI, ml/m ²	0.919	0.875 - 0.964	0.0005
RV EF, %	0.964	0.939 - 0.990	0.007
RV Mass, g/m ²	0.996	0.979 - 1.013	0.64
LV EDVI, ml/m ²	0.936	0.906 - 0.968	<0.0001
LV SVI, ml/m ²	0.923	0.887 - 0.959	<0.0001
LV EF, %	0.987	0.961 - 1.013	0.32
LV Mass g/m ²	0.984	0.969 - 0.996	0.04
VMI	1.890	0.789 - 4.526	0.15
LAV, ml/m ²	1.008	0.980 - 1.037	0.58

CTD-PAH, connective tissue disease associated pulmonary arterial hypertension; 6MWD, six minute walk distance; FEV, forced expiratory volume in 1s; FVC, forced vital capacity; TLCO, transfer factor for the lung for carbon monoxide; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; RV, right ventricle; EDVI, end-diastolic volume index; SVI, stroke volume index; EF, ejection fraction; VMI, ventricular mass index; LAV, left atrial volume

5.3.5.2 Predictors of Survival at Multivariate Analysis

At multivariate analysis right ventricular stroke volume index remained an independent predictor of survival after correction for age, TLCO and the presence of connective-tissue disease (vs idiopathic PAH) (Table 5.5). The model was limited to four variables due to the total number of events (35) during the follow-up period.

Table 5.5 Multivariate mortality predictors

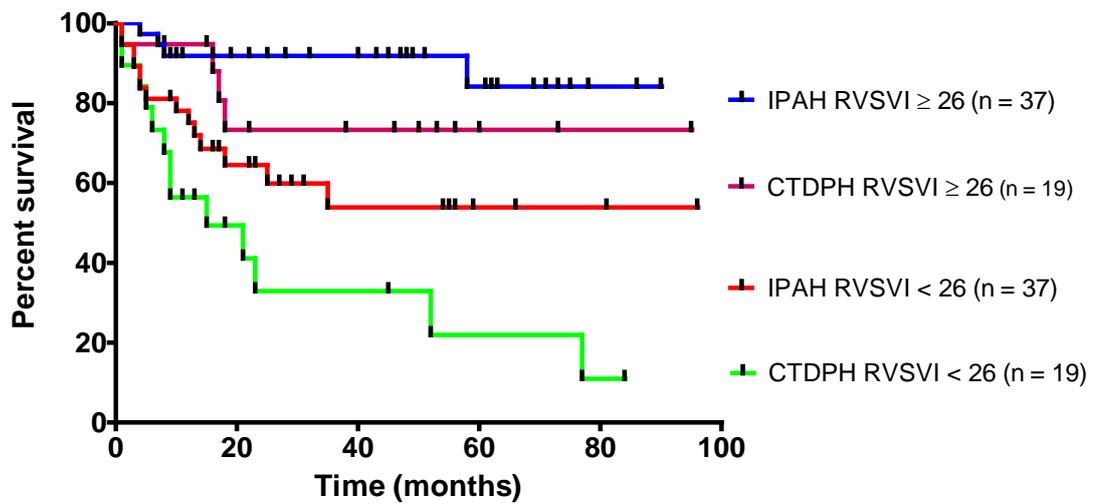
<u>Variable</u>	<u>Hazard Ratio</u>	<u>95% C.I.</u>	<u>P Value</u>
Age, yrs	1.044	1.008-1.082	0.02
TLCO (%)	0.968	0.939-0.998	0.04
Disease (CTD-PAH)	2.624	1.263-5.449	0.01
RV SVI, ml/m ²	0.927	0.880-0.977	0.005

TLCO, transfer factor for the lung for carbon monoxide; CTD-PAH, connective tissue disease associated pulmonary arterial hypertension; RVSVI, right ventricular stroke volume index

5.3.5.3 Survival by Disease and Baseline Right Ventricular Function

The study groups were stratified by disease and by baseline right ventricular function, either supramedian RV stroke volume index (≥ 26 ml/m²) or inframedian RV stroke volume index (< 26 ml/m²). Kaplan-Meier analysis was performed and the results presented in Figure 5.2. In IPAH, survival was better with higher RVSVI than with lower RVSVI ($p = 0.004$). Similarly, in CTD-PAH survival was better with higher RVSVI than with lower RVSVI ($p = 0.005$). Impaired right ventricular function at baseline, as measured by RVSVI, was associated with increased mortality in both IPAH and CTD-PAH.

Figure 5.2 Kaplan Meier survival estimates of the study cohort stratified by diagnosis and RV stroke volume index



Poorer right ventricular function (inframedian RVSVI) at baseline was associated with impaired survival in both IPAH and CTDPH-PAH.

IPAH, idiopathic pulmonary arterial hypertension; CTDPH-PAH, connective-tissue disease associated pulmonary arterial hypertension; RVSVI, right ventricular stroke volume index

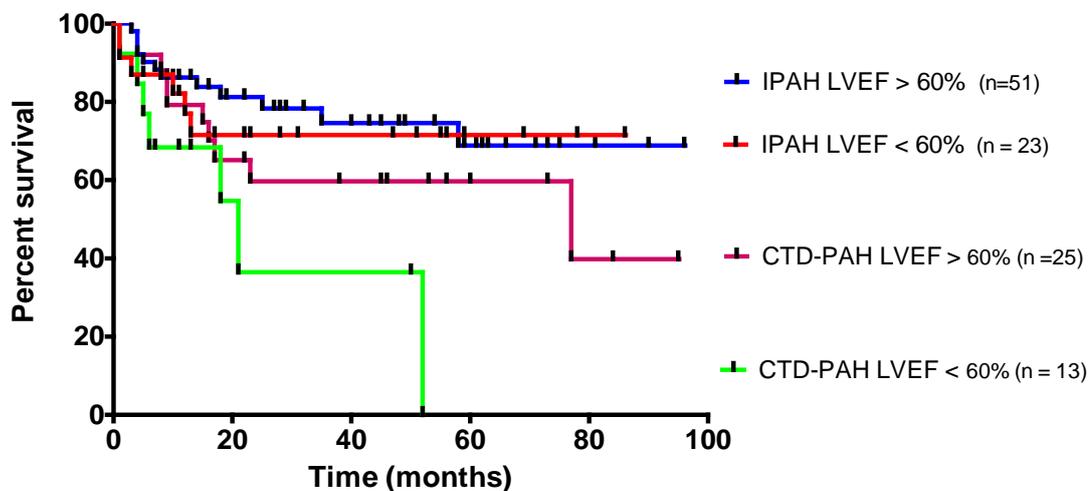
5.3.5.4 Survival by Disease and Baseline Left Ventricular Function

To investigate the importance of left ventricular function on survival, the study groups were also stratified by disease and by left ventricular systolic function, into normal LV ejection fraction ($> 60\%$) or reduced LV ejection fraction ($< 60\%$) (Figure 5.3).

Using this threshold, there was impaired LV systolic function in twenty-three out of the seventy-four (31%) IPAH patients, and in thirteen out of the thirty-eight (34%) CTD-PAH patients. In IPAH there was no difference in survival between patients with normal and reduced LV function ($p = 0.65$), however in CTD-PAH there was a trend towards poorer survival with reduced LV function which approached but did not fully reach statistical significance ($p = 0.09$).

The only measurement of left ventricular diastolic function available for the whole study group was CMR-derived left atrial volume (LAV), however when used alone it did not predict survival.

Figure 5.3 Kaplan Meier survival estimates of the study cohort stratified by diagnosis and LV ejection fraction



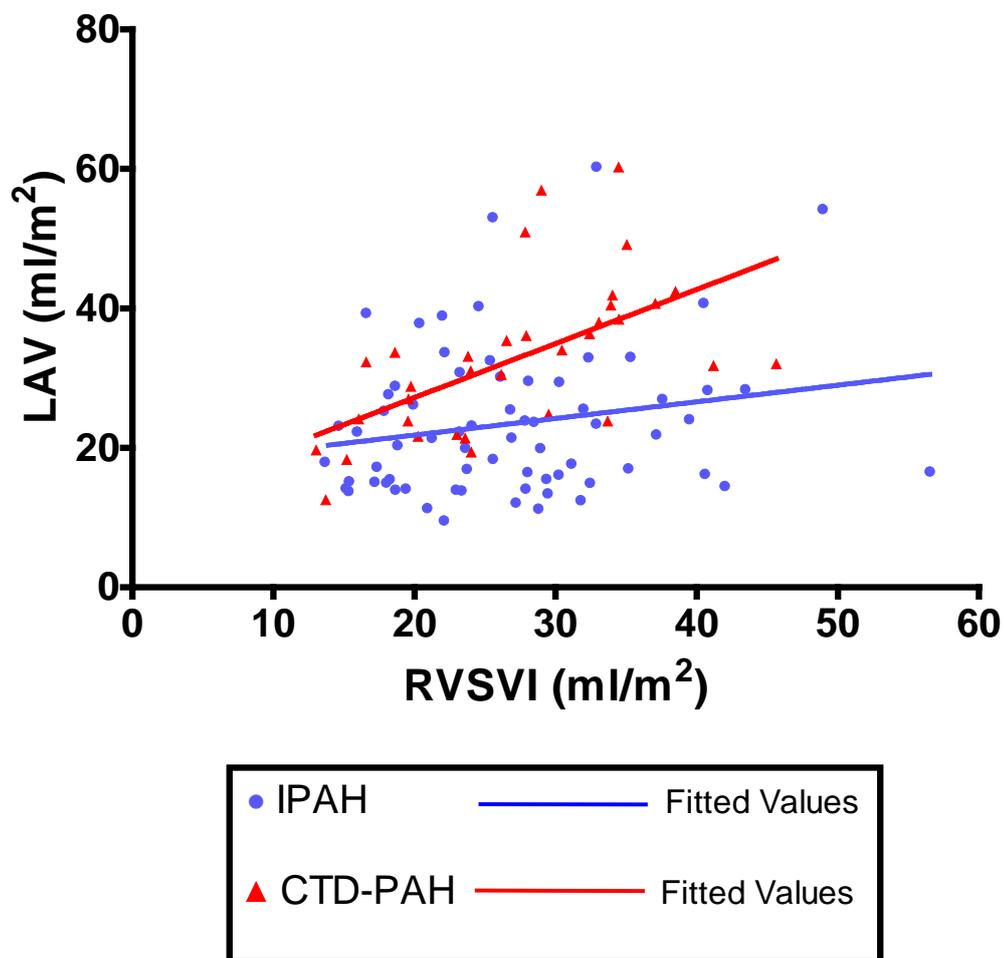
Survival rates of patients stratified according to disease, and into normal or reduced LV ejection fraction (LVEF). An LVEF < 60% was not associated with reduced survival in IPAH ($p = 0.65$), but there was a trend towards reduced survival in CTD-PAH ($p = 0.09$).

IPAH, idiopathic pulmonary arterial hypertension; CTD-PAH, connective-tissue disease associated pulmonary arterial hypertension

5.3.5.5 Relationship between Right Ventricular Filling and Left Atrial Volume

Linear regression analysis was performed to investigate whether the relationship between right ventricular filling (as measured by RVSVI) and left atrial volume was the same in IPAH as in CTD-PAH (Figure 5.4). With increasing RVSVI, left atrial volume increased more in CTD-PAH than in IPAH ($p = 0.035$ for the hypothesis of the slopes of the graph being equal). For a given RV stroke volume index, left atrial volume was higher in CTD-PAH than in IPAH.

Figure 5.4 Linear regression analysis of RV stroke volume index versus left atrial volume in IPAH and CTD-PAH



Relationship between left atrial volume (LAV) and right ventricular stroke volume index (RVSVI) is different in CTD-PAH ($r = 0.58$) than in IPAH ($r = 0.20$)

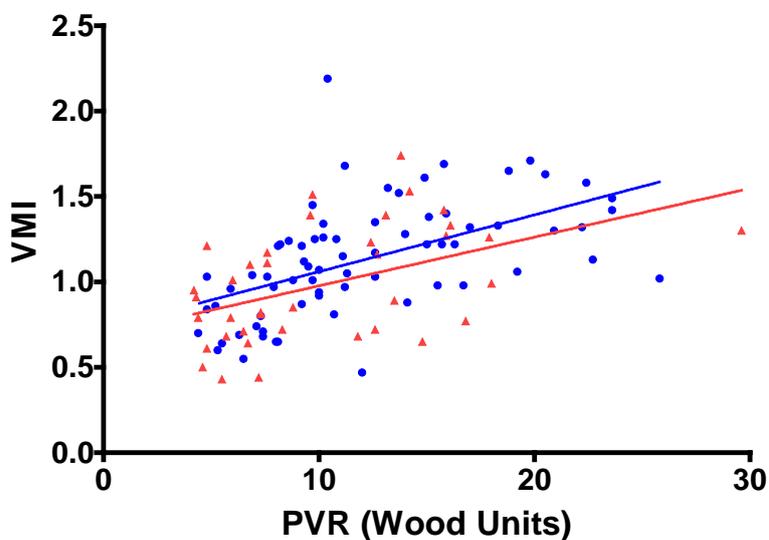
IPAH: idiopathic pulmonary arterial hypertension; CTD-PAH, connective tissue disease-associated pulmonary arterial hypertension

5.3.5.6 Relationship between right ventricular hypertrophy and pulmonary vascular resistance

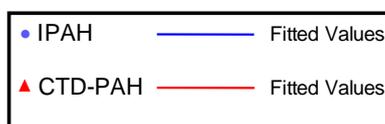
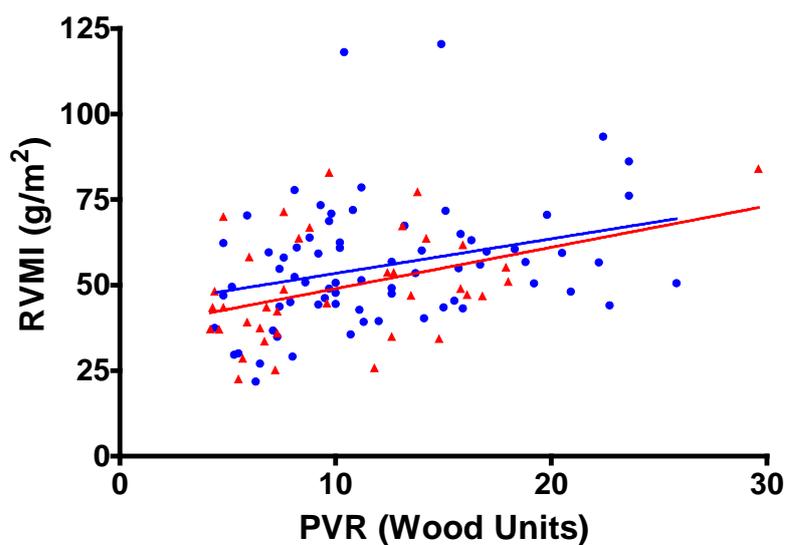
Linear regression analysis was performed to investigate whether the relationship between right ventricular hypertrophy and pulmonary vascular resistance was the same in IPAH as in CTD-PAH (Figure 5.5). There was no difference between IPAH and CTD-PAH in the relationship between VMI and PVR ($p = 0.66$ for the hypothesis of the slopes being equal), and no difference in the relationship between RV mass index alone and PVR between the two disease states ($p = 0.75$ for the hypothesis of the slopes being equal).

Figure 5.5 Linear regression analysis of PVR versus right ventricular hypertrophy in IPAH and CTD-PAH

(A)



(B)



Ventricular mass index (VMI) (A) and right ventricular mass index (B) versus pulmonary vascular resistance (PVR) stratified by diagnosis.

IPAH: idiopathic pulmonary arterial hypertension; CTD-PAH connective tissue disease associated pulmonary arterial hypertension

5.4 Discussion

The current study investigated survival differences between IPAH and CTD-PAH, with particular focus on the prognostic importance of right ventricular and left ventricular function. It has demonstrated that despite similar baseline demographics, lung function, haemodynamics, and PAH therapy, survival in CTD-PAH is much worse than in IPAH. Poor right ventricular function at baseline is associated with increased mortality in both conditions, but left ventricular function may also have some prognostic significance in CTD-PAH.

The findings of the current study support the recent interest in the hypothesis that right ventricular abnormalities may contribute to the poorer survival in CTD-PAH. Right ventricular dysfunction is common in CTD-PAH, with 37% of patients in the REVEAL registry having mild/moderate RV systolic dysfunction at echocardiography and 19% having severe RV systolic dysfunction¹⁷³, although these were actually lower rates than those observed in the IPAH group (mild/moderate 47%, severe 24%). At multivariate analysis, specific components of right ventricular dysfunction (RHC-derived stroke volume index and pulmonary arterial capacitance) have been found to contribute to increased mortality in SSc-PAH³²⁷.

Overbeek *et al* compared the relationship between mean RV pressure and stroke volume in SSc-PAH and IPAH, and demonstrated that patients with SSc-PAH had lower stroke volumes for any given mean RV pressure, suggesting impaired RV contractility in SSc-PAH³²⁸. Tedford *et al* also used pressure-volume loops to demonstrate that RV dysfunction is worse in SSc-PAH compared with IPAH at similar afterload, proposing that it may be due to problems with intrinsic RV systolic function rather than enhanced pulmonary vascular resistive and pulsatile loading³²⁹.

In a study comparing CMR-derived RV Mass and VMI with RHC-derived PVR, patients with SSc-PAH were found to have significantly less RV hypertrophy with increasing PVR than patients with IPAH³³⁰. The conclusion from Kelemen *et al* was that this difference in adaptive hypertrophy could explain decreased contractility and poorer survival in SSc-PAH. By contrast, in the current study there was no difference in the relationship of right ventricular hypertrophy to pulmonary vascular resistance between the two disease states of IPAH and CTD-PAH. The reason for these differing results is unclear but may relate to the use of PAH-specific therapy at the time of cardiac catheterisation and CMR. The

population in the current study was entirely treatment-naïve, whereas in the Kelemen group 100% of the IPAH patients but only 37% of the SSc-PAH patients were on PAH-specific medications.

There are two potential mechanisms by which PAH therapy could have affected the observed relationships between RV mass and PVR. Firstly, PAH-specific medications could have led to a reduction in RV hypertrophy in certain patients unrelated to changes in PVR. Earlier investigations have shown that short-term use of PDE-5 inhibitors but not endothelin antagonists can reduce RV mass^{293, 303}. Secondly, despite PAH therapy, RV mass may have remained fixed, but treatment has resulted in a reduction in PVR, and therefore a change in the relationship between RV hypertrophy and RV load. Longer-term studies of PAH therapy, have not demonstrated a sustained change in RV mass after treatment^{115, 118, 302, 331}. The current findings suggest the relationship between RV hypertrophy and RV load is similar between the disease states of IPAH and CTD-PAH at baseline, but may be modified by the use of PAH-specific therapies, with subsequent maladaptive hypertrophy and poorer outcome occurring in patients with underlying connective-tissue disease.

Whilst the majority of the patients diagnosed with IPAH and CTD-PAH in this study had normal left ventricular systolic function (mean LVEF of 63% in IPAH and 64% in CTD-PAH), about a third of patients in each group had an LVEF at CMR of <60%. A reduced LVEF was not of prognostic significance in IPAH, but was possibly associated with impaired survival in CTD-PAH, although this did not fully reach statistical significance. The reason for this prognostic difference may relate to differences in the likely aetiology of reduced LV function between the conditions.

In idiopathic PAH, impairment of LV function at the time of diagnosis is likely to be secondary to right ventricular abnormalities and ventricular interdependence, such as abnormal septal motion with impaired LV filling^{41, 42}. The normal anatomic spiral arrangement of the interventricular septum results in a twisting action that contributes to the forceful ejection of blood from both ventricles during systole, and therefore abnormal septal motion from elevated RV pressure can reduce ejection fraction from both the LV and RV^{332, 333}. Echocardiography with speckle tracking has shown that RV dyssynchrony is significantly associated with LV dyssynchrony and reduced LV ejection fraction in patients with pulmonary hypertension³³⁴. CMR has also been used to demonstrate reduced LV longitudinal and circumferential contractility in PAH³³⁵, reduced LV ejection fraction

in the setting of emphysema and right ventricular hypertrophy³³⁶, and delayed contrast enhancement in the interventricular septum of patients with PH¹³⁰. Even with normal LV size and normal conventional measures of LV systolic function, patients with PAH have been found to have reduced LV free wall systolic strain, and this was associated with delayed relaxation mitral inflow Doppler pattern and increased mortality³³⁷. It is reasonable to expect these left-sided abnormalities to improve as RV function improves following the commencement of PAH-specific therapy, and the recent longitudinal EURO-MR study demonstrated a small but statistically significant increase in LV ejection fraction after twelve months of PAH treatment³³¹.

In CTD-PAH, particularly when related to systemic sclerosis, impaired LV function at baseline may also relate to direct effects of the underlying connective-tissue disease on the left heart³³⁸, and these abnormalities may not resolve despite improvements in RV function after PAH therapy. It is well recognized that patients with systemic sclerosis have a high prevalence of left-sided cardiac abnormalities. In a small CMR study of 52 patients with systemic sclerosis, Hachulla *et al* demonstrated that 75% of the study cohort had at least one CMR abnormality³³⁹, including thinning of LV myocardium, impaired LV or RV ejection fractions, a pericardial effusion or LV diastolic dysfunction (35% of patients). Pericardial effusions occurred more frequently in CTD-PAH than in IPAH, but may be a manifestation of inflammatory serositis in some patients¹⁷³.

In a large multicentre French study in which echocardiography was used to prospectively screen 570 patients with SSc for PAH, LV systolic dysfunction was rare (1.4%) but LV hypertrophy (22.6%) and LV diastolic dysfunction (17.7%) were common³³⁸. The presence of left ventricular diastolic dysfunction at echocardiography has been associated with increased mortality in systemic sclerosis^{340, 341}. Left ventricular abnormalities in systemic sclerosis, particularly diastolic dysfunction, may predate clinical symptoms by several years³⁴². Post-mortem studies have demonstrated cardiac involvement in 70% of scleroderma patients compared with 37% of age and sex matched controls, however this was often clinically silent³⁴³. In their recent retrospective review, Fox *et al* found that 11/29 scleroderma patients with mPAP >25 mmHg and PAWP ≤ 15 mmHg were unmasked as having “occult pulmonary venous hypertension” by measurement of LV end-diastolic pressure (LVEDP) ± fluid challenge³⁴⁴. However, they were not able to determine if these patients had (Group 2) pulmonary venous hypertension, or had genuine PAH and concomitant diastolic dysfunction.

In the current study, left atrial volume was found to be higher in CTD-PAH than in IPAH, supporting the hypothesis that there may be coexisting LV diastolic dysfunction in CTD-PAH. In addition, patients with CTD-PAH had a higher LAV for a given RV stroke volume index than those with IPAH. In LV diastolic dysfunction there is abnormal LV relaxation and increased LV filling pressure²⁴⁹, which can result in enlargement of the thin-walled left atrium, allowing left atrial volume to be used as a marker of the severity and chronicity of diastolic dysfunction^{206, 250-253}. When used as a single variable, a decreased LAV was not found to be associated with better survival, however this might relate to the fact that a low LAV can also be the result of impaired filling from a failing right ventricle.

One of the main novel findings from this chapter is that RV function appears to have a similar impact on prognosis in CTD-PAH as seen in IPAH. Patients with CTD-PAH and poor RV function at baseline have very poor survival, supporting the hypothesis that RV failure is the major determinant of outcome in CTD-PAH. It is not clear from the current data whether this is in part due to a global cardiomyopathy, but the increased LA size observed in CTD-PAH and the potential significant impact of LV function raise this possibility. CMR has identified a subpopulation at an exceptionally high risk of death, and future work may include using CMR data to guide disease-targeted therapy in these patients.

There are some limitations of the current study that should be identified. CMR rather than echocardiography was used as the main imaging modality, and it is not possible to include E/e' (early transmitral filling (E) to early diastolic mitral annular velocity (e')) data or other measurements of left ventricular diastolic function³⁴⁵. Using an LVEF threshold of 60% may have stratified some patients with "low-normal" LV function into the "reduced LV systolic function group", although the threshold was applied to both IPAH and CTD-PAH cohorts. The retrospective nature of the current study meant it was not possible to use data from the more advanced CMR techniques that are helping to substantially develop our knowledge of left heart disease in connective-tissue disease. These include late gadolinium enhancement, tissue phase mapping and more detailed LV dynamic assessment^{346, 347}.

In the current study all-cause mortality was examined, rather than specifically death from right heart failure, however survival rates in both IPAH and CTD-PAH were similar to

those observed in the recent large UK registries^{185, 299}. The observed mortality rates and fairly small sample size meant it was not possible to include more than 3 or 4 variables in the multivariate analysis, and the duration of followup was smaller than in typical survival studies. By including patients with PAH due to connective-tissue diseases other than systemic sclerosis, there was heterogeneity within the CTD-PAH group that would not be observed if investigating SSc-PAH alone. In a small minority of patients it was not possible to determine the exact underlying connective-tissue disease, however the diagnosis of CTD-PAH was ascribed at a contemporaneous MDT. The intention was to exclude patients with underlying lung disease from the study, however given that observed TLCO was significantly lower in both IPAH and CTD-PAH than is commonly seen in these cohorts^{185, 299}, it raises the possibility that some patients with lung disease were included in the final analysis. Information was available on the initial PAH therapy commenced at diagnosis, but not on subsequent changes to the PAH treatment regime during the follow-up period.

In this chapter it has been shown that survival is worse in CTD-PAH than in IPAH, despite similar baseline characteristics, lung function, pulmonary haemodynamics and initial PAH therapy. Poor right ventricular function at baseline was associated with markedly reduced survival in CTD-PAH, supporting the hypothesis that RV function is the main determinant of outcome in this condition. Reduced left ventricular systolic function was associated with poorer survival in CTD-PAH but not in IPAH, and patients with CTD-PAH had higher LAV raising the possibility of LV diastolic dysfunction in this group. Left heart disease may contribute to the poorer survival observed in CTD-PAH, possibly as part of a global cardiomyopathy, so the diagnostic assessment of CTD-PAH should include close interrogation of left ventricular function.

Chapter 6

Major Findings and Conclusions

6. Major Findings and Conclusions

The work in this thesis was undertaken to evaluate the role of cardiac magnetic resonance imaging in the diagnosis and determination of outcome in pulmonary hypertension.

The ability of CMR to distinguish IPAH from PH-HFpEF was investigated, with particular focus on the assessment of left atrial volume. Distinguishing IPAH from PH-HFpEF has become an area of great importance over recent years as the incidence of HFpEF, with subsequent PH-HFpEF, is increasing annually. There has been progress in distinguishing isolated post-capillary PH from combined pre-capillary and post-capillary PH in HFpEF, along with growing awareness of PAH-specific therapies and enthusiasm for their use in PH-HFpEF. Concurrently, the average age at diagnosis of IPAH is increasing, with recent registry data suggesting it is now a disease of middle-aged and older people. PAH-specific therapies are expensive, potentially harmful in left heart disease, and current practice is to distinguish between these conditions with invasive RHC.

In Chapter 3, left atrial volume measured using CMR was a sensitive, specific and non-invasive method for distinguishing IPAH from PH-HFpEF. 36 out of 37 patients with IPAH had a LAV of less than 43 ml/m^2 , and all the PH-HFpEF patients had a LAV of more than 43 ml/m^2 . The threshold of 43 ml/m^2 was obtained through ROC analysis, and it would be important in future study to prospectively apply this threshold to a cohort of patients undergoing diagnostic assessment for PH, allowing evaluation of CMR based prediction of RHC findings and the overall utility of CMR in reaching the diagnostic conclusion.

LAV was assessed using CMR in the Scottish Pulmonary Vascular Unit, the only specialist PH centre in Scotland. Future work could be undertaken to identify if left atrial volume assessment performed at non-specialist centres, perhaps with echocardiography, is of similar utility. It would also be useful to investigate the relationship between CMR-LAV and other echocardiographic measures of left ventricular diastolic function in IPAH and PH-HFpEF. CMR may have a future role in distinguishing between HFpEF with “passive” PH versus “reactive” PH, particularly given the increased interest in the use of PAH therapies in the latter group.

The last two decades have witnessed a vast expansion in the therapeutic options available to treat PAH, ranging from intravenous epoprostenol to oral monotherapy or upfront combination therapy. These treatments are typically approved on the basis of improvements in pulmonary haemodynamics or modest increases in exercise capacity, and less is known about their overall effect on the RV. CMR is already established as the gold standard for the evaluation of RV structure and function during the diagnostic assessment of PAH.

In Chapter 4, CMR was used to demonstrate improvements in right ventricular function after four and twelve months of disease-targeted PAH therapy. This corresponded to the improvements observed in NYHA functional class and exercise capacity, and was observed in both the PDE-5 and ERA treated groups. This study included both patients with PAH, and those with PH due to other conditions including hypoxic lung disease and chronic thromboembolic disease. Whilst this heterogeneity reflects everyday clinical practice in pulmonary vascular medicine, it may be useful in future work to recruit a more homogenous PAH population, to minimize the impact of coexisting respiratory disease or other comorbidities. However, as PAH therapies are being increasingly used in Group 3-5 PH, it would also be important to determine if similar improvements in RV function are being achieved in these patients, and additional separate CMR studies would be required to achieve this. Assuming a treatment-induced change in RVSVI of 4.89 ml/m^2 and a SD of 10.5 ml/m^2 based on the findings in Chapter 4, the minimum sample size required to detect a treatment effect with 90% power and 5% two-tailed level of significance test would be 49 patients.

In this study PAH therapy was commenced and modified at the discretion of the local PH physician, resulting in significant treatment variation between centres. Future multi-centre studies would likely benefit from a more standardized treatment algorithm, and this could potentially allow CMR to be used to investigate the impact of specific drug therapies on the RV. It is clear from the baseline characteristics of the study groups in Chapter 4 that there is significant variability in the patient populations between centres, with those in Glasgow having the poorest functional class, lowest 6MWD and most severe pulmonary haemodynamics. Despite this, the results observed within the Glasgow subgroup reflected the findings observed for the cohort as a whole, supporting the hypothesis that disease-targeted therapies can improve RV function in both moderate and severe PH. There has been significant expansion in the therapeutic options available in PAH since this study was

conceived, with greater use of combination therapy and the development of oral prostanoids, soluble guanylate cyclase activators and newer endothelin antagonists and PDE-5 inhibitors. It would be essential that any future CMR studies investigating RV function reflected contemporary clinical practice and included patients receiving these newer treatments.

Clinical outcomes were not routinely measured in this multi-centre study, so future work may involve relating on-treatment changes in RV function at CMR with a clinically predefined outcome, typically a combination of mortality or need for intravenous therapy. This would clearly increase the size, cost and complexity of such a study, and mandate the need for proper study oversight, but would increase the clinical significance and validity of the study findings. It is likely that a prolonged follow-up period, well beyond the 12 months of the current work, would be required for this, thereby providing an opportunity to investigate the impact of long-term PAH therapy on the RV. It would also be important to take advantage of the more advanced CMR techniques now available for assessment of RV structure function and the pulmonary circulation. These include pulmonary artery stiffness, right ventricular-arterial coupling or transverse shortening, and assessment of PH patients during exercise or dobutamine administration. CMR techniques used in the longitudinal assessment of RV function in PAH need to have excellent interstudy reproducibility, and this could be further investigated beyond the current analysis by way of a test-retest study.

The poor survival observed in CTD-PAH, especially when compared with IPAH, has been an area of longstanding concern in the pulmonary vascular community. Proposed explanations have included delayed diagnosis, poorer tolerance of PAH therapies, and the effect of coexisting cardiac or respiratory disease.

In Chapter 5, it was observed that survival in CTD-PAH was significantly poorer than in IPAH, despite similar clinical characteristics, pulmonary haemodynamics, PAH treatments, and the exclusion of patients with interstitial lung disease. Using CMR, RV function at the time of diagnosis was found to be an important predictor of survival in both conditions, with impaired RV function in CTD-PAH associated with a particularly poor outcome. The findings of the current study support the concept that RV failure is the main determinant of outcome in CTD-PAH, and highlight the need for further research into optimal treatment

strategies for these patients, potentially including specific therapies directed at the right ventricle. The relationship between PVR and the degree of RV hypertrophy at baseline was the same in both conditions, but future research could determine if this altered after the introduction of PAH therapy. This single centre study had a relatively small sample size and short duration of follow-up, limiting the extent of multivariate analysis, so it would be beneficial for future studies to have a larger number of patients, potentially across different PH centres, and a more prolonged follow-up period. It would also be important to ensure that strict entry criteria were in place to minimize the risk of patients with underlying lung disease being included in the study.

In the current study there was evidence to support the hypothesis of left ventricular problems contributing to the increased mortality in CTD-PAH, and this is in keeping with recent progress in our understanding of left heart disease in CTD. Compared with IPAH, patients with CTD-PAH had higher LAV, a higher increase in LAV for a given RV stroke volume, and LV systolic dysfunction was more commonly associated with reduced survival. In the current study it was not possible to comprehensively evaluate LV systolic and diastolic function in all patients, but this should now be considered during the routine diagnostic assessment of suspected CTD-PAH. If this study was being repeated then more detailed CMR evaluation of LV function would be invaluable, and could help determine if a global cardiomyopathy is contributing to increased mortality in CTD-PAH.

Appendix 1 Patient Consent Form and Information Sheet

WEST ETHICS COMMITTEE

FORM OF CONSENT FOR PATIENTS/VOLUNTEERS IN CLINICAL RESEARCH PROJECT

Title of Project: The Assessment of Pulmonary Hypertension using Cardiac MRI

By signing this form you give consent to your participation in the project whose title is at the top of the page. You should have been given a complete explanation of the project to your satisfaction and have been given the opportunity to ask questions. You should also have been given a copy of the patient information sheet approved by the West Ethics Committee to read and to keep. Even though you have agreed to take part in the research procedures you may withdraw this consent at any time without the need to explain why and without any prejudice to your care.

Consent:

.....(PRINT)

of

give my consent to the research procedures above, the nature, purpose and possible consequences of which have been described to me

by

Patient's signatureDate.....

Doctor's signature

INFORMATION SHEET FOR PATIENTS/VOLUNTEERS IN CLINICAL RESEARCH PROJECT

Brief Title of Project

The Assessment of Pulmonary Hypertension using Cardiac MRI

Patient's Summary (Purpose of study, nature of procedure, discomfort and possible risks in terms which the patient or volunteer can understand).

Background

Pulmonary Hypertension is a rare and potentially serious condition. It is often difficult to diagnose and to treat. The major problem in Pulmonary Hypertension is high blood pressure in the lungs; this often leads to excessive stress being exerted on the right hand side of the heart. Sometimes, as a result of this, the right side of the heart becomes unable to pump blood into the lungs, as it should. This obviously can make the patient feel very unwell.

We usually make the diagnosis after a number of tests have been carried out; the most important of these currently is a Right Heart Catheter. This involves passing a plastic catheter into the heart through a vein in the neck or groin. Through this catheter we can measure the blood pressure in the lungs and assess how well the heart is pumping. We can also assess how likely the patient is to respond to treatment.

This test is very useful and generally safe, but some patients find it unpleasant and there are some risks involved. We therefore do not tend to repeat it to see how well or badly a patient is doing unless it is absolutely necessary. Instead, we use a test that measures how far a patient can walk in six minutes. We then compare this measurement to the last one to say whether they are better or worse. This test is useful but can sometimes be misleading.

The Study

We are conducting a study looking at new ways to diagnose and monitor patients with Pulmonary Hypertension. We would like to invite you to take part. The study involves the use of a relatively new scanning technique called MRI (Magnetic Resonance Imaging).

MRI allows us to get a lot of useful information about Pulmonary Hypertension. It does not use any X-rays or radiation, but instead uses magnets to acquire detailed images of pulmonary blood vessels and the heart. It is therefore very safe. We can get a lot of the same information using MRI that we get at right heart catheterisation.

Compared with right heart catheterisation, MRI is safer, and patients tend to find it less unpleasant. This means not only could MRI be used in the diagnosis of Pulmonary Hypertension, we would be happier to repeat it in the future. This may allow us to better identify changes in the illness over time and so allow better tailoring of your treatment.

In addition to the MRI we would like to measure certain proteins in your bloodstream that may go up and down depending on how well you are.

What the study involves

During your 5-day stay at the Western Infirmary you will have a number of tests, including blood samples and x-rays. On the Thursday you will have your right heart catheterisation. We would propose doing an MRI scan at some time during this week. The scan would take around 1½ hours and would not prolong your stay in hospital. We will take some extra blood samples in addition (around one tablespoon in total). These samples will be taken off at the same time as your routine bloods and will not involve any extra needles. We normally see patients about 3 months after their discharge in clinic and repeat the six-minute walking test. You will follow this normal routine. **As part of the study you will also have further MRI scans 4 months and 12 months later**, to assess your progress. If required, we will be able to organise for a taxi to pick you up and take you home free of charge.

What does the MRI involve?

On arrival at the Clinical Research Initiative (CRI) a radiographer will go through a safety checklist and make sure that all magnetic objects (e.g. jewellery and bankcards) have been removed. Following this you will be asked to sign the consent form. Once you have changed into a hospital gown you will then be asked to lie flat on the bed that will move into the scanner. The scanner is basically tunnel shaped, like a large “polo” mint. You are slid into the centre of the “polo” on an electric bed and the scans are acquired. Some patients find it a little enclosing, but you can come out at any time.

When you are in the scanner you will need to wear a pair of headphones, allowing you to listen to music of your choice (you are welcome to bring your own CD) and to allow us to communicate with you throughout the scan. The headphones are also necessary because of the loud knocking noise that occurs when the pictures are being taken. You will be given an emergency buzzer and you can very quickly be taken out the scanner should you feel it uncomfortable or if it is felt necessary. During the scan you will be asked to hold your breath at times to improve the quality of the pictures.

What are the risks?

◦ The MRI scanner is very safe as long as you have no metal implants in your body.

- NB**
- You should not take part in this study if you are pregnant.
 - If you wish to take part in this study, your General Practitioner will be advised of your participation and the clinical management that you will undergo.
 - If you do not wish to participate in this study, or wish to withdraw at any time your care will not, in any way, be affected.

If you have any questions regarding the study please contact the Scottish Pulmonary Vascular Unit on 0141 211 1812.

Appendix 2 World Health Organisation Functional Class

- Functional class I** Patients with PH but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near syncope.
- Functional class II** Patients with PH resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope.
- Functional class III** Patients with PH resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope.
- Functional class IV** Patients with PH with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity.

References

1. Dalonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT, Levy PS, Pietra GG, Reid LM, Reeves JT, Rich S, Vreim CE, Williams GW, Wu M. Survival in patients with primary pulmonary-hypertension - results from a national prospective registry. *Ann. Intern. Med.* 1991;115:343-349
2. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G. Guidelines for the diagnosis and treatment of pulmonary hypertension. *European Respiratory Journal.* 2009;34:1219-1263
3. Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, Gibbs S, Lebrec D, Speich R, Beghetti M, Rich S, Fishman A. Clinical classification of pulmonary hypertension. *Journal of the American College of Cardiology.* 2004;43:5S-12S
4. Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, Elliott CG, Gaine SP, Gladwin MT, Jing ZC, Krowka MJ, Langleben D, Nakanishi N, Souza R. Updated clinical classification of pulmonary hypertension. *Journal of the American College of Cardiology.* 2009;54:S43-S54
5. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, Sanchez MAG, Kumar RK, Landzberg M, Machado RF, Olschewski H, Robbins IM, Souza R. Updated clinical classification of pulmonary hypertension. *Journal of the American College of Cardiology.* 2013;62:D34-D41
6. Tuder RM. Pathology of pulmonary arterial hypertension. *Seminars in Respiratory and Critical Care Medicine.* 2009;30:376-385
7. Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, Reid LM, Tuder RM. Pathologic assessment of vasculopathies in pulmonary hypertension. *Journal of the American College of Cardiology.* 2004;43:25S-32S
8. Hassoun PM, Mouthon L, Barbera JA, Eddahibi S, Flores SC, Grimminger F, Jones PL, Maitland ML, Michelakis ED, Morrell NW, Newman JH, Rabinovitch M, Schermuly R, Stenmark KR, Voelkel NF, Yuan JXJ, Humbert M. Inflammation, growth factors, and pulmonary vascular remodeling. *Journal of the American College of Cardiology.* 2009;54:S10-S19
9. Morrell NW, Adnot S, Archer SL, Dupuis J, Jones PL, MacLean MR, McMurtry IF, Stenmark KR, Thistlethwaite PA, Weissmann N, Yuan JXJ, Weir EK. Cellular and molecular basis of pulmonary arterial hypertension. *Journal of the American College of Cardiology.* 2009;54:S20-S31
10. Humbert M, Morrell NW, Archer SL, Stenmark KR, MacLean MR, Lang IM, Christman BW, Weir EK, Eickelberg O, Voelkel NF, Rabinovitch M. Cellular and molecular pathobiology of pulmonary arterial hypertension. *Journal of the American College of Cardiology.* 2004;43:13S-24S
11. Fedullo PF, Auger WR, Kerr KM, Rubin LJ. Current concepts: Chronic thromboembolic pulmonary hypertension. *New England Journal of Medicine.* 2001;345:1465-1472
12. Pepke-Zaba J, Delcroix M, Lang I, Mayer E, Jansa P, Ambroz D, Treacy C, D'Armini AM, Morsolini M, Snijder R, Bresser P, Torbicki A, Kristensen B, Lewczuk J, Simkova I, Barbera JA, de Perrot M, Hoeper MM, Gaine S, Speich R, Gomez-Sanchez MA, Kovacs G, Hamid AM, Jais X, Simonneau G. Chronic thromboembolic pulmonary hypertension (cteph) results from an international prospective registry. *Circulation.* 2011;124:1973-1981

13. Lang IM. Chronic thromboembolic pulmonary hypertension - not so rare after all. *New England Journal of Medicine*. 2004;350:2236-2238
14. Lee FA. Hemodynamics of the right ventricle in normal and disease states. *Cardiology Clinics*. 1992;10:59-68
15. Haddad F, Hunt SA, Rosenthal DN, Murphy DJ. Right ventricular function in cardiovascular disease, part i - anatomy, physiology, aging, and functional assessment of the right ventricle. *Circulation*. 2008;117:1436-1448
16. Ho SY, Nihoyannopoulos P. Anatomy, echocardiography, and normal right ventricular dimensions. *Heart*. 2006;92:12-113
17. Dell'Italia LJ. The right ventricle: Anatomy, physiology, and clinical importance. *Current problems in cardiology*. 1991;16:653-720
18. Farrerbr.G, Rowles PM. Vascular supply of interventricular septum of human heart. *British Heart Journal*. 1969;31:727-&
19. Farb A, Burke AP, Virmani R. Anatomy and pathology of the right ventricle including acquired tricuspid and pulmonic valve disease. *Cardiology Clinics*. 1992;10:1-22
20. Anderson RH, Sanchez-Quintana D, Niederer P, Lunkenheimer PP. Structural-functional correlates of the 3-dimensional arrangement of the myocytes making up the ventricular walls. *Journal of Thoracic and Cardiovascular Surgery*. 2008;136:10-18
21. Buckberg G. Cardiac structure/function relationships: Reality or deduction? *Journal of Thoracic and Cardiovascular Surgery*. 2008;136:19-20
22. Castella M, Buckberg GD, Saleh S, Gharib M. Structure function interface with sequential shortening of basal and apical components of the myocardial band. *European Journal of Cardio-Thoracic Surgery*. 2005;27:980-987
23. Chin KM, Kim NHS, Rubin LJ. The right ventricle in pulmonary hypertension. *Coronary Artery Disease*. 2005;16:13-18
24. Burgess MI, Mogulkoc N, Bright-Thomas RJ, Bishop P, Egan JJ, Ray SG. Comparison of echocardiographic markers of right ventricular function in determining prognosis in chronic pulmonary disease. *Journal of the American Society of Echocardiography*. 2002;15:633-639
25. Macnee W. Pathophysiology of cor-pulmonale in chronic obstructive pulmonary-disease .1. *American Journal of Respiratory and Critical Care Medicine*. 1994;150:833-852
26. Haddad F, Doyle R, Murphy DJ, Hunt SA. Right ventricular function in cardiovascular disease, part ii - pathophysiology, clinical importance, and management of right ventricular failure. *Circulation*. 2008;117:1717-1731
27. Davlouros PA, Niwa K, Webb G, Gatzoulis MA. The right ventricle in congenital heart disease. *Heart*. 2006;92:127-138
28. Messika-Zeitoun D, Thomson H, Bellamy M, Scott C, Tribouilloy C, Dearani J, Tajik AJ, Schaff H, Enriquez-Sarano M. Medical and surgical outcome of tricuspid regurgitation caused by flail leaflets. *Journal of Thoracic and Cardiovascular Surgery*. 2004;128:296-302
29. Goldhaber SZ, Visani L, De Rosa M, Icooper. Acute pulmonary embolism: Clinical outcomes in the international cooperative pulmonary embolism registry (icoper). *Lancet*. 1999;353:1386-1389
30. Dias CA, Assad RS, Caneo LF, Abduch MCD, Aiello VD, Dias AR, Marcial MB, Oliveira SA. Reversible pulmonary trunk banding. Ii. An experimental model for rapid pulmonary ventricular hypertrophy. *Journal of Thoracic and Cardiovascular Surgery*. 2002;124:999-1006
31. Chen EP, Craig DM, Bittner HB, Davis RD, Van Trigt P. Pharmacological strategies for improving diastolic dysfunction in the setting of chronic pulmonary hypertension. *Circulation*. 1998;97:1606-1612

32. Louie EK, Lin SS, Reynertson SI, Brundage BH, Levitsky S, Rich S. Pressure and volume loading of the right ventricle have opposite effects on left-ventricular ejection fraction. *Circulation*. 1995;92:819-824
33. Hopkins WE, Ochoa LL, Richardson GW, Trulock EP. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or eisenmenger syndrome. *Journal of Heart and Lung Transplantation*. 1996;15:100-105
34. Hopkins WE, Waggoner AD. Severe pulmonary hypertension without right ventricular failure: The unique hearts of patients with eisenmenger syndrome. *American Journal of Cardiology*. 2002;89:34-38
35. Hopkins WE. The remarkable right ventricle of patients with eisenmenger syndrome. *Coronary Artery Disease*. 2005;16:19-25
36. Voelkel NF, Quaife RA, Leinwand LA, Barst RJ, McGoon MD, Meldrum DR, Dupuis J, Long CS, Rubin LJ, Smart FW, Suzuki YJ, Gladwin M, Denholm EM, Gail DB. Right ventricular function and failure - report of a national heart, lung, and blood institute working group on cellular and molecular mechanisms of right heart failure. *Circulation*. 2006;114:1883-1891
37. Handoko ML, de Man FS, Allaart CP, Paulus WJ, Westerhof N, Vonk-Noordegraaf A. Perspectives on novel therapeutic strategies for right heart failure in pulmonary arterial hypertension: Lessons from the left heart. *Eur Respir Rev*. 2010;19:72-82
38. Bogaard HJ, Abe K, Noordegraaf AV, Voelkel NF. The right ventricle under pressure cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest*. 2009;135:794-804
39. Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeier TJ, Quaife RA, Roden RL, Dutcher DL, Robertson AD, Voelkel NF, Badesch DB, Groves BM, Gilbert EM, Bristow MR. Changes in gene expression in the intact human heart - downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *Journal of Clinical Investigation*. 1997;100:2315-2324
40. Vonk-Noordegraaf A, Marcus JT, Gan CT, Boonstra A, Postmus PE. Interventricular mechanical asynchrony due to right ventricular pressure overload in pulmonary hypertension plays an important role in impaired left ventricular filling. *Chest*. 2005;128:628S-630S
41. Marcus JT, Gan CTJ, Zwanenburg JJM, Boonstra A, Allaart CP, Gbttte MJW, Vonk-Noordegraaf A. Interventricular mechanical asynchrony in pulmonary arterial hypertension. *Journal of the American College of Cardiology*. 2008;51:750-757
42. Gan CTJ, Lankhaar JW, Marcus JT, Westerhof N, Marques KM, Bronzwaer JGF, Boonstra A, Postmus PE, Vonk-Noordegraaf A. Impaired left ventricular filling due to right-to-left ventricular interaction in patients with pulmonary arterial hypertension. *American Journal of Physiology-Heart and Circulatory Physiology*. 2006;290:H1528-H1533
43. Gurudevan SV, Malouf PJ, Auger WR, Waltman TJ, Madani M, Raisinghani AB, DeMaria AN, Blanchard DG. Abnormal left ventricular diastolic filling in chronic thromboembolic pulmonary hypertension - true diastolic dysfunction or left ventricular underfilling? *Journal of the American College of Cardiology*. 2007;49:1334-1339
44. Ciarka A, Vachiery JL, Houssiere A, Gujic M, Stoupel E, Velez-Roa S, Naeije R, van de Borne P. Atrial septostomy decreases sympathetic overactivity in pulmonary arterial hypertension. *Chest*. 2007;131:1831-1837
45. Velez-Roa S, Ciarka A, Najem B, Vachiery JL, Naeije R, van de Borne P. Increased sympathetic nerve activity in pulmonary artery hypertension. *Circulation*. 2004;110:1308-1312

46. Kramer MR, Valantine HA, Marshall SE, Starnes VA, Theodore J. Recovery of the right ventricle after single-lung transplantation in pulmonary-hypertension. *American Journal of Cardiology*. 1994;73:494-500
47. Toyooka S, Kusano KF, Goto K, Masaomi Y, Oto T, Sano Y, Fuke S, Okazaki M, Ohe T, Kasahara S, Sano S, Date H. Right but not left ventricular function recovers early after living-donor lobar lung transplantation in patients with pulmonary arterial hypertension. *Journal of Thoracic and Cardiovascular Surgery*. 2009;138:222-226
48. Come PC, Kim D, Parker JA, Goldhaber SZ, Braunwald E, Markis JE. Early reversal of right ventricular dysfunction in patients with acute pulmonary-embolism after treatment with intravenous tissue plasminogen-activator. *Journal of the American College of Cardiology*. 1987;10:971-978
49. Fukui S, Ogo T, Morita Y, Tsuji A, Tateishi E, Ozaki K, Sanda Y, Fukuda T, Yasuda S, Ogawa H, Nakanishi N. Right ventricular reverse remodelling after balloon pulmonary angioplasty. *European Respiratory Journal*. 2014;43:1394-1402
50. Rolf A, Rixe J, Kim WK, Borgel J, Mollmann H, Nef HM, Liebetrau C, Kramm T, Guth S, Krombach GA, Mayer E, Hamm CW. Right ventricular adaptation to pulmonary pressure load in patients with chronic thromboembolic pulmonary hypertension before and after successful pulmonary endarterectomy - a cardiovascular magnetic resonance study. *Journal of Cardiovascular Magnetic Resonance*. 2014;16:7
51. McLaughlin VV, Sitbon O, Badesch DB, Barst RJ, Black C, Galle N, Rainisio M, Simonneau G, Rubin LJ. Survival with first-line bosentan in patients with primary pulmonary hypertension. *European Respiratory Journal*. 2005;25:244-249
52. Sandoval J, Bauerle O, Palomar A, Gomez A, Martinezguerra ML, Beltran M, Guerrero L. Survival in primary pulmonary-hypertension - validation of a prognostic equation. *Circulation*. 1994;89:1733-1744
53. Sitbon O, Humbert M, Nunes H, Parent F, Garcia G, Herve P, Rainisio M, Simonneau G. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension - prognostic factors and survival. *Journal of the American College of Cardiology*. 2002;40:780-788
54. Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK, Levy PC, Reid LM, Vreim CE, Williams GW. Primary pulmonary-hypertension - a national prospective-study. *Ann. Intern. Med*. 1987;107:216-223
55. Gaine SP, Rubin LJ. Primary pulmonary hypertension. *Lancet*. 1998;352:719-725
56. Tongers J, Schwerdtfeger B, Klein G, Kempf T, Schaefer A, Knapp JM, Niehaus M, Korte T, Hoepfer MM. Incidence and clinical relevance of supraventricular tachyarrhythmias in pulmonary hypertension. *American Heart Journal*. 2007;153:127-132
57. Thenappan T, Shah SJ, Gomberg-Maitland M, Collander B, Vallakati A, Shroff P, Rich S. Clinical characteristics of pulmonary hypertension in patients with heart failure and preserved ejection fraction. *Circulation: Heart Failure*. 2011;4:257-265
58. Tan RT, Kuzo R, Goodman LR, Siegel R, Haasler GB, Presberg KW, Med Coll Wisconsin Lung Transplant G. Utility of ct scan evaluation for predicting pulmonary hypertension in patients with parenchymal lung disease. *Chest*. 1998;113:1250-1256
59. Groves AM, Win T, Charman SC, Wisbey C, Pepke-Zaba J, Coulden RA. Semi-quantitative assessment of tricuspid regurgitation on contrast-enhanced multidetector ct. *Clinical Radiology*. 2004;59:715-719
60. Roberts HC, Kauczor HU, Schweden F, Thelen M. Spiral ct of pulmonary hypertension and chronic thromboembolism. *Journal of Thoracic Imaging*. 1997;12:118-127

61. Shimizu H, Tanabe N, Terada J, Masuda M, Sakao S, Kasahara Y, Takiguchi Y, Tatsumi K, Kuriyama T. Dilatation of bronchial arteries correlates with extent of central disease in patients with chronic thromboembolic pulmonary hypertension. *Circulation Journal*. 2008;72:1136-1141
62. Heinrich M, Uder M, Tscholl D, Grgic A, Kramann B, Schafers HJ. Ct scan findings in chronic thromboembolic pulmonary hypertension - predictors of hemodynamic improvement after pulmonary thromboendarterectomy. *Chest*. 2005;127:1606-1613
63. Worsley DF, Palevsky HI, Alavi A. Ventilation-perfusion lung-scanning in the evaluation of pulmonary-hypertension. *Journal of Nuclear Medicine*. 1994;35:793-796
64. Tunariu N, Gibbs SJR, Win Z, Gin-Sing W, Graham A, Gishen P, Al-Nahhas A. Ventilation-perfusion scintigraphy is more sensitive than multidetector ctpa in detecting chronic thromboembolic pulmonary disease as a treatable cause of pulmonary hypertension. *Journal of Nuclear Medicine*. 2007;48:680-684
65. Hoepfer MM, Mayer E, Simonneau G, Rubin LJ. Chronic thromboembolic pulmonary hypertension. *Circulation*. 2006;113:2011-2020
66. Widera E, Sulica R. Pulmonary artery sarcoma misdiagnosed as chronic thromboembolic pulmonary hypertension. *Mount Sinai Journal of Medicine*. 2005;72:360-364
67. Rich S, Kieras K, Hart K, Groves BM, Stobo JD, Brundage BH. Antinuclear antibodies in primary pulmonary-hypertension. *Journal of the American College of Cardiology*. 1986;8:1307-1311
68. Fisher MR, Forfia PR, Chamera E, Houston-Harris T, Champion HC, Girgis RE, Corretti MC, Hassoun PM. Accuracy of doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2009;179:615-621
69. Forfia PR, Fisher MR, Mathai SC, Houston-Harris T, Hemnes AR, Borlaug BA, Chamera E, Corretti MC, Champion HC, Abraham TP, Girgis RE, Hassoun PM. Tricuspid annular displacement predicts survival in pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2006;174:1034-1041
70. Mathai SC, Sibley CT, Forfia PR, Mudd JO, Fisher MR, Tedford RJ, Lechtzin N, Boyce D, Hummers LK, Houston T, Zaiman AL, Girgis RE, Hassoun PM. Tricuspid annular plane systolic excursion is a robust outcome measure in systemic sclerosis-associated pulmonary arterial hypertension. *Journal of Rheumatology*. 2011;38:2410-2418
71. Eysmann SB, Palevsky HI, Reichel N, Hackney K, Douglas PS. Two-dimensional and doppler-echocardiographic and cardiac-catheterization correlates of survival in primary pulmonary-hypertension. *Circulation*. 1989;80:353-360
72. Raymond RJ, Hinderliter AL, Willis PW, Ralph D, Caldwell EJ, Williams W, Ettinger NA, Hill NS, Summer WR, de Boisblanc B, Schwartz T, Koch G, Clayton LM, Jobsis MM, Crow JW, Long W. Echo cardiographic predictors of adverse outcomes in primary pulmonary hypertension. *Journal of the American College of Cardiology*. 2002;39:1214-1219
73. Tei C, Dujardin KS, Hodge DO, Bailey KR, McGoon MD, Tajik AJ, Seward SB. Doppler echocardiographic index for assessment of global right ventricular function. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 1996;9:838-847
74. Yeo TC, Dujardin KS, Tei CW, Mahoney DW, McGoon MD, Seward JB. Value of a doppler-derived index combining systolic and diastolic time intervals in predicting outcome in primary pulmonary hypertension. *American Journal of Cardiology*. 1998;81:1157-1161

75. Brooks D, Solway S. Ats statement on six-minute walk test. *American Journal of Respiratory and Critical Care Medicine*. 2003;167:1287-1287
76. Crapo RO, Casaburi R, Coates AL, Enright PL, MacIntyre NR, McKay RT, Johnson D, Wanger JS, Zeballos RJ, Bittner V, Mottram C, Comm ATS. Ats statement: Guidelines for the six-minute walk test. *American Journal of Respiratory and Critical Care Medicine*. 2002;166:111-117
77. Lee WTN, Peacock AJ, Johnson MK. The role of per cent predicted 6-min walk distance in pulmonary arterial hypertension. *European Respiratory Journal*. 2010;36:1294-1301
78. Frost AE, Langleben D, Oudiz R, Hill N, Horn E, McLaughlin V, Robbins IM, Shapiro S, Tapson VF, Zwicke D, DeMarco T, Schilz R, Rubenfire M, Barst RJ, Grp S-S. The 6-min walk test (6mw) as an efficacy endpoint in pulmonary arterial hypertension clinical trials: Demonstration of a ceiling effect. *Vascular Pharmacology*. 2005;43:36-39
79. Galie N, Rubin LJ, Hoeper MM, Jansa P, Al-Hiti H, Meyer GMB, Chiossi E, Kusic-Pajic A, Simonneau G. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (early study): A double-blind, randomised controlled trial. *Lancet*. 2008;371:2093-2100
80. Degano B, Sitbon O, Savale L, Garcia G, O'Callaghan DS, Jais X, Humbert M, Simonneau G. Characterization of pulmonary arterial hypertension patients walking more than 450 m in 6 min at diagnosis. *Chest*. 2010;137:1297-1303
81. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JFO, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in france - results from a national registry. *American Journal of Respiratory and Critical Care Medicine*. 2006;173:1023-1030
82. Miyamoto S, Nagaya N, Satoh T, Kyotani S, Sakamaki F, Fujita M, Nakanishi N, Miyatake K. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension - comparison with cardiopulmonary exercise testing. *American Journal of Respiratory and Critical Care Medicine*. 2000;161:487-492
83. Savarese G, Paolillo S, Costanzo P, D'Amore C, Cecere M, Losco T, Musella F, Gargiulo P, Marciano C, Perrone-Filardi P. Do changes of 6-minute walk distance predict clinical events in patients with pulmonary arterial hypertension? A meta-analysis of 22 randomized trials. *Journal of the American College of Cardiology*. 2012;60:1192-1201
84. Macchia A, Marchioli R, Tognoni G, Scarano M, Marfisi R, Tavazzi L, Rich S. Systematic review of trials using vasodilators in pulmonary arterial hypertension: Why a new approach is needed. *American Heart Journal*. 2010;159:13
85. Mathai SC, Puhan MA, Lam D, Wise RA. The minimal important difference in the 6-minute walk test for patients with pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2012;186:428-433
86. Wensel R, Opitz CF, Anker SD, Winkler J, Hoffken G, Kleber FX, Sharma R, Hummel M, Hetzer R, Ewert R. Assessment of survival in patients with primary pulmonary hypertension importance of cardiopulmonary exercise testing. *Circulation*. 2002;106:319-324
87. Sackner MA, Greenelch D, Heiman MS, Epstein S, Atkins N. Diffusing-capacity, membrane diffusing-capacity, capillary blood-volume, pulmonary tissue volume, and cardiac-output measured by a rebreathing technique. *American Review of Respiratory Disease*. 1975;111:157-165
88. Lang CC, Karlin P, Haythe J, Tsao L, Mancini DM. Ease of noninvasive measurement of cardiac output coupled with peak vo2 determination at rest and

- during exercise in patients with heart failure. *American Journal of Cardiology*. 2007;99:404-405
89. Corte TJ, Wells AU, Gatzoulis MA, Cramer D, Ward S, Macdonald PS, Dimopoulos K, Wort SJ. Non-invasive assessment of pulmonary blood flow using an inert gas rebreathing device in fibrotic lung disease. *Thorax*. 2010;65:341-345
 90. McLure LER, Brown A, Lee WN, Church AC, Peacock AJ, Johnson MK. Non-invasive stroke volume measurement by cardiac magnetic resonance imaging and inert gas rebreathing in pulmonary hypertension. *Clinical Physiology and Functional Imaging*. 2011;31:221-226
 91. Yandle TG. Biochemistry of natriuretic peptides. *Journal of Internal Medicine*. 1994;235:561-576
 92. Blyth KG, Groenning BA, Mark PB, Martin TN, Foster JE, Steedman T, Morton JJ, Dargie HJ, Peacock AJ. Nt-probnp can be used to detect right ventricular systolic dysfunction in pulmonary hypertension. *European Respiratory Journal*. 2007;29:737-744
 93. Gan CT, McCann GP, Marcus JT, van Wolferen SA, Twisk JW, Boonstra A, Postmus PE, Vonk-Noordegraf A. Nt-probnp reflects right ventricular structure and function in pulmonary hypertension. *European Respiratory Journal*. 2006;28:1190-1194
 94. Andreassen AK, Wergeland R, Simonsen S, Geiran O, Guevara C, Ueland T. N-terminal pro-b-type natriuretic peptide as an indicator of disease severity in a heterogeneous group of patients with chronic precapillary pulmonary hypertension. *American Journal of Cardiology*. 2006;98:525-529
 95. Williams MH, Handler CE, Akram R, Smith CJ, Das C, Smee J, Nair D, Denton CP, Black CM, Coghlan JG. Role of n-terminal brain natriuretic peptide (nt-probnp) in scleroderma-associated pulmonary arterial hypertension. *European Heart Journal*. 2006;27:1485-1494
 96. Leuchte HH, Holzapfel M, Neurohr C, Vogeser M, Behr J. Characterization of brain natriuretic peptide in long-term follow-up of pulmonary arterial hypertension. *Chest*. 2005;128:2368-2374
 97. Nagaya N, Ando M, Oya M, Ohkita Y, Kyotani S, Sakamaki F, Nakanishi N. Plasma brain natriuretic peptide as a noninvasive marker for efficacy of pulmonary thromboendarterectomy. *Annals of Thoracic Surgery*. 2002;74:180-184
 98. Swan HJC, Ganz W, Forrester J, Marcus H, Diamond G, Chonette D. Catheterization of heart in man with use of flow-directed balloon-tipped catheter. *New England Journal of Medicine*. 1970;283:447-&
 99. Frost AE, Farber HW, Barst RJ, Miller DP, Elliott CG, McGoon MD. Demographics and outcomes of patients diagnosed with pulmonary hypertension with pulmonary capillary wedge pressures 16 to 18 mm hg insights from the reveal registry. *Chest*. 2013;143:185-195
 100. Robbins IM, Hemnes AR, Pugh ME, Brittain EL, Zhao DX, Piana RN, Fong PP, Newman JH. High prevalence of occult pulmonary venous hypertension revealed by fluid challenge in pulmonary hypertension. *Circulation-Heart Failure*. 2014;7:116-122
 101. Sitbon O, Humbert M, Jais X, Ioos V, Hamid AM, Provencher S, Garcia G, Parent F, Herve P, Simonneau G. Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. *Circulation*. 2005;111:3105-3111
 102. Dalen JE. The pulmonary artery catheter - friend, foe, or accomplice? *Jama-Journal of the American Medical Association*. 2001;286:348-350
 103. Evans DC, Doraiswamy VA, Prosciak MP, Silveira M, Seamon MJ, Funes VR, Cipolla J, Wang CF, Kavuturu S, Torigian DA, Cook CH, Lindsey DE, Steinberg SM, Stawicki SP. Complications associated with pulmonary artery catheters: A comprehensive clinical review. *Scandinavian Journal of Surgery*. 2009;98:199-208

104. Kearney TJ, Shabot MM. Pulmonary-artery rupture associated with the swan-ganz catheter. *Chest*. 1995;108:1349-1352
105. Caldini P, Gensini GG, Hoffman MS. Primary pulmonary hypertension with death during right heart catheterization - case report and a survey of reported fatalities. *American Journal of Cardiology*. 1959;4:519-527
106. Fuster V, Steele PM, Edwards WD, Gersh BJ, McGoon MD, Frye RL. Primary pulmonary-hypertension - natural-history and the importance of thrombosis. *Circulation*. 1984;70:580-587
107. Hoepfer MM, Lee SH, Voswinckel R, Palazzini M, Jais X, Marinelli A, Barst RJ, Ghofrani HA, Jing ZC, Opitz C, Seyfarth HJ, Halank M, McLaughlin V, Oudiz RJ, Ewert R, Wilkens H, Kluge S, Bremer HC, Baroke E, Rubin LJ. Complications of right heart catheterization procedures in patients with pulmonary hypertension in experienced centers. *Journal of the American College of Cardiology*. 2006;48:2546-2552
108. Ley S, Kreitner KF, Fink C, Heussel CP, Borst MM, Kauczor HU. Assessment of pulmonary hypertension by ct and mr imaging. *European Radiology*. 2004;14:359-368
109. McLure LER, Peacock AJ. Cardiac magnetic resonance imaging for the assessment of the heart and pulmonary circulation in pulmonary hypertension. *European Respiratory Journal*. 2009;33:1454-1466
110. Longmore DB, Klipstein RH, Underwood SR, Firmin DN, Hounsfield GN, Watanabe M, Bland C, Fox K, Poolewilson PA, Rees RSO, Denison D, McNeilly AM, Burman ED. Dimensional accuracy of magnetic-resonance in studies of the heart. *Lancet*. 1985;1:1360-1362
111. Bottini PB, Carr AA, Prisant LM, Flickinger FW, Allison JD, Gottdiener JS. Magnetic-resonance-imaging compared to echocardiography to assess left-ventricular mass in the hypertensive patient. *American Journal of Hypertension*. 1995;8:221-228
112. Grothues F, Smith GC, Moon JCC, Bellenger NG, Collins P, Klein HU, Pennell DJ. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *American Journal of Cardiology*. 2002;90:29-34
113. Grothues F, Moon JC, Bellenger NG, Smith GS, Klein HU, Pennell DJ. Interstudy reproducibility of right ventricular volumes, function, and mass with cardiovascular magnetic resonance. *American Heart Journal*. 2004;147:218-223
114. Bradlow WM, Hughes ML, Keenan NG, Bucciarelli-Ducci C, Assomull R, Gibbs JSR, Mohiaddin RH. Measuring the heart in pulmonary arterial hypertension (pah): Implications for trial study size. *Journal of Magnetic Resonance Imaging*. 2010;31:117-124
115. Roeleveld RJ, Vonk-Noordegraaf A, Marcus JT, Bronzwaer JGF, Marques KMJ, Postmus PE, Boonstra A. Effects of epoprostenol on right ventricular hypertrophy and dilatation in pulmonary hypertension. *Chest*. 2004;125:572-579
116. Hoepfer MM, Tongers J, Leppert A, Baus S, Maier R, Lotz J. Evaluation of right ventricular performance with a right ventricular ejection fraction thermodilution catheter and mri in patients with pulmonary hypertension. *Chest*. 2001;120:502-507
117. Marcus JT, Noordegraaf AV, Roeleveld RJ, Postmus PE, Heethaar RM, Van Rossum AC, Boonstra A. Impaired left ventricular filling due to right ventricular pressure overload in primary pulmonary hypertension - noninvasive monitoring using mri. *Chest*. 2001;119:1761-1765
118. van Wolferen SA, Marcus JT, Boonstra A, Marques KMJ, Bronzwaer JGF, Spreeuwenberg MD, Postmus PE, Vonk-Noordegraaf A. Prognostic value of right

- ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. *European Heart Journal*. 2007;28:1250-1257
119. van Wolferen SA, van de Veerdonk MC, Mauritz GJ, Jacobs W, Marcus JT, Marques KMJ, Bronzwaer JGE, Heymans MW, Boonstra A, Postmus PE, Westerhof N, Noordegraaf AV. Clinically significant change in stroke volume in pulmonary hypertension. *Chest*. 2011;139:1003-1009
 120. Katz J, Whang J, Boxt LM, Barst RJ. Estimation of right ventricular mass in normal subjects and in patients with primary pulmonary-hypertension by nuclear-magnetic-resonance imaging. *Journal of the American College of Cardiology*. 1993;21:1475-1481
 121. Marcus JT, Noordegraaf AV, De Vries P, Van Rossum AC, Roseboom B, Heethaar RM, Postmus PE. Mri evaluation of right ventricular pressure overload in chronic obstructive pulmonary disease. *Journal of Magnetic Resonance Imaging*. 1998;8:999-1005
 122. Saba TS, Foster J, Cockburn M, Cowan M, Peacock AJ. Ventricular mass index using magnetic resonance imaging accurately estimates pulmonary artery pressure. *European Respiratory Journal*. 2002;20:1519-1524
 123. Hagger D, Condliffe R, Woodhouse N, Elliot CA, Armstrong IJ, Davies C, Hill C, Akil M, Wild JM, Kiely DG. Ventricular mass index correlates with pulmonary artery pressure and predicts survival in suspected systemic sclerosis-associated pulmonary arterial hypertension. *Rheumatology*. 2009;48:1137-1142
 124. King ME, Braun H, Goldblatt A, Liberthson R, Weyman AE. Interventricular septal configuration as a predictor of right ventricular systolic hypertension in children - a cross-sectional echocardiographic study. *Circulation*. 1983;68:68-75
 125. Ricciardi MJ, Bossone E, Bach DS, Armstrong WF, Rubenfire M. Echocardiographic predictors of an adverse response to a nifedipine trial in primary pulmonary hypertension - diminished left ventricular size and leftward ventricular septal bowing. *Chest*. 1999;116:1218-1223
 126. Kasimir MT, Seebacher G, Jaksch P, Winkler G, Schmid K, Marta GM, Simon P, Klepetko W. Reverse cardiac remodelling in patients with primary pulmonary hypertension after isolated lung transplantation. *European Journal of Cardio-Thoracic Surgery*. 2004;26:776-781
 127. Reesink HJ, Marcus JT, Tulevski II, Jamieson S, Kloek JJ, Noordegraaf AV, Bresser P. Reverse right ventricular remodeling after pulmonary endarterectomy in patients with chronic thromboembolic pulmonary hypertension: Utility of magnetic resonance imaging to demonstrate restoration of the right ventricle. *Journal of Thoracic and Cardiovascular Surgery*. 2007;133:58-64
 128. Roeleveld RJ, Marcus JT, Faes TJC, Gan TJ, Boonstra A, Postmus PE, Vonk-Noordegraaf A. Interventricular septal configuration at mr imaging and pulmonary arterial pressure in pulmonary hypertension. *Radiology*. 2005;234:710-717
 129. Mauritz GJ, Kind T, Marcus JT, Bogaard HJ, van de Veerdonk M, Postmus PE, Boonstra A, Westerhof N, Vonk-Noordegraaf A. Progressive changes in right ventricular geometric shortening and long-term survival in pulmonary arterial hypertension. *Chest*. 2012;141:935-943
 130. Blyth KG, Groenning BA, Martin TN, Foster JE, Mark PB, Dargie HJ, Peacock AJ. Contrast enhanced-cardiovascular magnetic resonance imaging in patients with pulmonary hypertension. *European Heart Journal*. 2005;26:1993-1999
 131. Gerges M, Gerges C, Lang IM. Advanced imaging tools rather than hemodynamics should be the primary approach for diagnosing, following, and managing pulmonary arterial hypertension. *Canadian Journal of Cardiology*. 2015;31:521-528
 132. Roeleveld RJ, Marcus JT, Boonstra A, Postmus PE, Marques KM, Bronzwaer JGF, Vonk-Noordegraaf A. A comparison of noninvasive mri-based methods of

- estimating pulmonary artery pressure in pulmonary hypertension. *Journal of Magnetic Resonance Imaging*. 2005;22:67-72
133. Laffon E, Vallet C, Bernard V, Montaudon M, Ducassou D, Laurent F, Marthan R. A computed method for noninvasive mri assessment of pulmonary arterial hypertension. *Journal of Applied Physiology*. 2004;96:463-468
 134. Lankhaar JW, Noordegraaf AV, Marcus JT. A computed method for noninvasive mri assessment of pulmonary arterial hypertension. *Journal of Applied Physiology*. 2004;97:794-794
 135. Garcia-Alvarez A, Fernandez-Friera L, Mirelis JG, Sawit S, Nair A, Kallman J, Fuster V, Sanz J. Non-invasive estimation of pulmonary vascular resistance with cardiac magnetic resonance. *European Heart Journal*. 2011;32:2438-2445
 136. Swift AJ, Rajaram S, Hurdman J, Hill C, Davies C, Sproson TW, Morton AC, Capener D, Elliot C, Condliffe R, Wild JM, Kiely DG. Noninvasive estimation of pa pressure, flow, and resistance with cmr imaging derivation and prospective validation study from the aspire registry. *Jacc-Cardiovascular Imaging*. 2013;6:1036-1047
 137. Mereles D, Ehlken N, Kreuscher S, Ghofrani S, Hoeper MM, Halank M, Meyer FJ, Karger G, Buss J, Juenger J, Holzapfel N, Opitz C, Winkler J, Herth FFJ, Wilkens H, Katus HA, Olschewski H, Grunig E. Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. *Circulation*. 2006;114:1482-1489
 138. Babu AS, Padmakumar R, Maiya AG. A review of ongoing trials in exercise based rehabilitation for pulmonary arterial hypertension. *Indian Journal of Medical Research*. 2013;137:900-906
 139. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Noordegraaf AV, Beghetti M, Ghofrani A, Sanchez MAG, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 esc/ers guidelines for the diagnosis and treatment of pulmonary hypertension. *European Respiratory Journal*. 2015;46:903-975
 140. Weitzenblum E, SautegEAU A, Ehrhart M, Mammosser M, Pelletier A. Long-term oxygen-therapy can reverse the progression of pulmonary-hypertension in patients with chronic obstructive pulmonary-disease. *American Review of Respiratory Disease*. 1985;131:493-498
 141. Ahmedzai S, Balfour-Lynn IM, Bewick T, Buchdahl R, Coker RK, Cummin AR, Gradwell DP, Howard L, Innes JA, Johnson AOC, Lim E, Lim WS, McKinlay KP, Partridge MR, Popplestone M, Pozniak A, Robson A, Shovlin CL, Shrikrishna D, Simonds A, Tait P, Thomas M, British Thoracic Soc S. Managing passengers with stable respiratory disease planning air travel: British thoracic society recommendations. *Thorax*. 2011;66:1-30
 142. Burns RM, Peacock AJ, Johnson MK, Church AC. Hypoxaemia in patients with pulmonary arterial hypertension during simulated air travel. *Respiratory Medicine*. 2013;107:298-304
 143. Herve P, Humbert M, Sitbon O, Parent F, Nunes H, Legal C, Garcia G, Simonneau G. Pathobiology of pulmonary hypertension - the role of platelets and thrombosis. *Clinics in Chest Medicine*. 2001;22:451-+
 144. Rich S, Seidlitz M, Dodin E, Osimani D, Judd D, Genthner D, McLaughlin V, Francis G. The short-term effects of digoxin in patients with right ventricular dysfunction from pulmonary hypertension. *Chest*. 1998;114:787-792
 145. Jones DA, Benjamin CW, Linseman DA. Activation of thromboxane and prostacyclin receptors elicits opposing effects on vascular smooth-muscle cell-growth and mitogen-activated protein-kinase signaling cascades. *Molecular Pharmacology*. 1995;48:890-896

146. Galie N, Manes A, Branzi A. Prostanoids for pulmonary arterial hypertension. *American journal of respiratory medicine : drugs, devices, and other interventions*. 2003;2:123-137
147. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension - the impact of epoprostenol therapy. *Circulation*. 2002;106:1477-1482
148. Simonneau G, Barst RJ, Galie N, Naeije R, Rich S, Bourge RC, Keogh A, Oudiz R, Frost A, Blackburn SD, Crow JW, Rubin LJ, Treprostinil Study G. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension - a double-blind, randomized, placebo-controlled trial. *American Journal of Respiratory and Critical Care Medicine*. 2002;165:800-804
149. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ. Expression of endothelin-1 in the lungs of patients with pulmonary-hypertension. *New England Journal of Medicine*. 1993;328:1732-1739
150. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovascular Research*. 2004;61:227-237
151. Channick RN, Simonneau G, Sitbon O, Robbins IM, Frost A, Tapson VF, Badesch DB, Roux S, Rainisio M, Bodin F, Rubin LJ. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: A randomised placebo-controlled study. *Lancet*. 2001;358:1119-1123
152. McGoon MD, Frost AE, Oudiz RJ, Badesch DB, Galie N, Olschewski H, McLaughlin VV, Gerber MJ, Dufton C, Despain DJ, Rubin LJ. Ambrisentan therapy in patients with pulmonary arterial hypertension who discontinued bosentan or sitaxsentan due to liver function test abnormalities. *Chest*. 2009;135:122-129
153. Pulido T, Adzerikho I, Channick RN, Delcroix M, Galie N, Ghofrani HA, Jansa P, Jing ZC, Le Brun FO, Mehta S, Mittelholzer CM, Perchenet L, Sastry BKS, Sitbon O, Souza R, Torbicki A, Zeng XF, Rubin LJ, Simonneau G, Investigators S. Macitentan and morbidity and mortality in pulmonary arterial hypertension. *New England Journal of Medicine*. 2013;369:809-818
154. Lee WTN, Kirkham N, Johnson MK, Lordan JL, Fisher AJ, Peacock AJ. Sitaxentan-related acute liver failure in a patient with pulmonary arterial hypertension. *European Respiratory Journal*. 2011;37:472-474
155. Wharton J, Strange JW, Moller GMO, Growcott EJ, Ren XH, Franklyn AP, Phillips SC, Wilkins MR. Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. *American Journal of Respiratory and Critical Care Medicine*. 2005;172:105-113
156. Tantini B, Manes A, Fiumana E, Pignatti C, Guarnieri C, Zannoli R, Branzi A, Galie N. Antiproliferative effect of sildenafil on human pulmonary artery smooth muscle cells. *Basic Research in Cardiology*. 2005;100:131-138
157. Ghofrani HA, Voswinckel R, Reichenberger F, Olschewski H, Haredza P, Karadas B, Schermuly RT, Weissmann N, Seeger W, Grimminger F. Differences in hemodynamic and oxygenation responses to three different phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension - a randomized prospective study. *Journal of the American College of Cardiology*. 2004;44:1488-1496
158. Bhatia S, Frantz RP, Severson CJ, Durst LA, McGoon MD. Immediate and long-term hemodynamic and clinical effects of sildenafil in patients with pulmonary arterial hypertension receiving vasodilator therapy. *Mayo Clinic Proceedings*. 2003;78:1207-1213

159. Michelakis ED, Tymchak W, Noga M, Webster L, Wu XC, Lien D, Wang SH, Modry D, Archer SL. Long-term treatment with oral sildenafil is safe and improves functional capacity and hemodynamics in patients with pulmonary arterial hypertension. *Circulation*. 2003;108:2066-2069
160. Ghofrani HA, Schermuly RT, Rose F, Wiedemann R, Kohstall MG, Kreckel A, Olschewski H, Weissmann N, Enke B, Ghofrani S, Seeger W, Grimminger F. Sildenafil for long-term treatment of nonoperable chronic thromboembolic pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2003;167:1139-1141
161. Khan AS, Sheikh Z, Khan S, Dwivedi R, Benjamin E. Viagra deafness-sensorineural hearing loss and phosphodiesterase-5 inhibitors. *Laryngoscope*. 2011;121:1049-1054
162. Grimminger F, Weimann G, Frey R, Voswinckel R, Thamm M, Bolkow D, Weissmann N, Muck W, Unger S, Wensing G, Schermuly RT, Ghofrani HA. First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension. *European Respiratory Journal*. 2009;33:785-792
163. Ghofrani HA, Galie N, Grimminger F, Grunig E, Humbert M, Jing ZC, Keogh AM, Langleben D, Kilama MO, Fritsch A, Neuser D, Rubin LJ. Riociguat for the treatment of pulmonary arterial hypertension. *New England Journal of Medicine*. 2013;369:330-340
164. Ghofrani HA, D'Armini AM, Grimminger F, Hoeper MM, Jansa P, Kim NH, Mayer E, Simonneau G, Wilkins MR, Fritsch A, Neuser D, Weimann G, Wang C. Riociguat for the treatment of chronic thromboembolic pulmonary hypertension. *New England Journal of Medicine*. 2013;369:319-329
165. Kawut SM, Taichman DB, Archer-Chicko CL, Palevsky HI, Kimmel SE. Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis. *Chest*. 2003;123:344-350
166. Alpert MA, Pressly TA, Mukerji V, Lambert CR, Mukerji B, Panayiotou H, Sharp GC. Acute and long-term effects of nifedipine on pulmonary and systemic hemodynamics in patients with pulmonary-hypertension associated with diffuse systemic-sclerosis, the crest syndrome and mixed connective-tissue disease. *American Journal of Cardiology*. 1991;68:1687-1691
167. Badesch DB, Tapon VF, McGoan MD, Brundage BH, Rubin LJ, Wigley FM, Rich S, Barst RJ, Barrett PS, Kral KM, Jobsis MM, Loyd JE, Murali S, Frost A, Girgis R, Bourge RC, Ralph DD, Elliott CG, Hill NS, Langleben D, Schilz RJ, McLaughlin VV, Robbins IM, Groves BM, Shapiro S, Medsger TA, Gaine SP, Horn E, Decker JC, Knobil K. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease - a randomized, controlled trial. *Ann. Intern. Med*. 2000;132:425-+
168. Oudiz RJ, Schilz RJ, Barst RJ, Galie N, Rich S, Rubin LJ, Simonneau G, Treprostinil Study G. Treprostinil, a prostacyclin analogue, in pulmonary arterial hypertension associated with connective tissue disease. *Chest*. 2004;126:420-427
169. Girgis RE, Mathai SC, Krishnan JA, Wigley FM, Hassoun PM. Long-term outcome of bosentan treatment in idiopathic pulmonary arterial hypertension and pulmonary arterial hypertension associated with the scleroderma spectrum of diseases. *Journal of Heart and Lung Transplantation*. 2005;24:1626-1631
170. Badesch DB, Hill NS, Burgess G, Rubin LJ, Barst RJ, Galie N, Simonneau G, Grp SS. Sildenafil for pulmonary arterial hypertension associated with connective tissue disease. *Journal of Rheumatology*. 2007;34:2417-2422
171. Avouac J, Wipff J, Kahan A, Allanore Y. Effects of oral treatments on exercise capacity in systemic sclerosis related pulmonary arterial hypertension: A meta-analysis of randomised controlled trials. *Annals of the Rheumatic Diseases*. 2008;67:808-814

172. Rubenfire M, Huffman MD, Krishnan S, Seibold JR, Schiopu E, McLaughlin VV. Survival in systemic sclerosis with pulmonary arterial hypertension has not improved in the modern era. *Chest*. 2013;144:1282-1290
173. Chung L, Liu J, Parsons L, Hassoun PM, McGoon M, Badesch DB, Miller DP, Nicolls MR, Zamanian RT. Characterization of connective tissue disease-associated pulmonary arterial hypertension from reveal identifying systemic sclerosis as a unique phenotype. *Chest*. 2010;138:1383-1394
174. Williams MH, Das C, Handler CE, Akram MR, Davar J, Denton CP, Smith CJ, Black CM, Coghlan JG. Systemic sclerosis associated pulmonary hypertension: Improved survival in the current era. *Heart*. 2006;92:926-932
175. Launay D, Sitbon O, Hachulla E, Mouthon L, Gressin V, Rottat L, Clerson P, Cordier JF, Simonneau G, Humbert M. Survival in systemic sclerosis-associated pulmonary arterial hypertension in the modern management era. *Annals of the Rheumatic Diseases*. 2013;72:1940-1946
176. Jais X, Launay D, Yaici A, Le Pavec J, Tcherakian C, Sitbon O, Simonneau G, Humbert M. Immunosuppressive therapy in lupus- and mixed connective tissue disease-associated pulmonary arterial hypertension. *Arthritis and Rheumatism*. 2008;58:521-531
177. Hoepfer MM, Barst RJ, Bourge RC, Feldman J, Frost AE, Galie N, Gomez-Sanchez MA, Grimminger F, Grunig E, Hassoun PM, Morrell NW, Peacock AJ, Satoh T, Simonneau G, Tapson VF, Torres F, Lawrence D, Quinn DA, Ghofrani HA. Imatinib mesylate as add-on therapy for pulmonary arterial hypertension results of the randomized impres study. *Circulation*. 2013;127:1128-+
178. Califf R, Adams K, McKenna W, Gheorghide M, Uretsky B, McNulty S, Darius H, Schulman K, Zannad F, HandbergThurmond E, Harrell F, Wheeler W, SolerSoler J, Swedberg K. A randomized controlled trial of epoprostenol therapy for severe congestive heart failure: The flolan international randomized survival trial (first). *American Heart Journal*. 1997:44-54
179. Kaluski E, Cotter G, Leitman M, Milo-Cotter O, Krakover R, Kobrin I, Moriconi T, Rainisio M, Caspi A, Reizin L, Zimlichman R, Vered Z. Clinical and hemodynamic effects of bosentan dose optimization in symptomatic heart failure patients with severe systolic dysfunction, associated with secondary pulmonary hypertension - a multi-center randomized study. *Cardiology*. 2008;109:273-280
180. Guazzi M, Vicenzi M, Arena R, Guazzi MD. Pulmonary hypertension in heart failure with preserved ejection fraction a target of phosphodiesterase-5 inhibition in a 1-year study. *Circulation*. 2011;124:164-U120
181. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, LeWinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, Felker GM, Goldsmith SR, Bart BA, McNulty SE, Ibarra JC, Lin G, Oh JK, Patel MR, Kim RJ, Tracy RP, Velazquez EJ, Anstrom KJ, Hernandez AF, Mascette AM, Braunwald E, Trial R. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction a randomized clinical trial. *Jama-Journal of the American Medical Association*. 2013;309:1268-1277
182. Bonderman D, Ghio S, Felix SB, Ghofrani H-A, Michelakis E, Mitrovic V, Oudiz RJ, Boateng F, Scalise A-V, Roessig L, Semigran MJ, Left Ventricular Systolic Dysfunction Associated With P, Hypertension Riociguat Trial Study G. Riociguat for patients with pulmonary hypertension caused by systolic left ventricular dysfunction: A phase iib double-blind, randomized, placebo-controlled, dose-ranging hemodynamic study. *Circulation*. 2013;128:502-511
183. Bonderman D, Pretsch I, Steringer-Mascherbauer R, Jansa P, Rosenkranz S, Tufaro C, Bojic A, Lam CSP, Frey R, Kilama MO, Unger S, Roessig L, Lang IM. Acute

- hemodynamic effects of riociguat in patients with pulmonary hypertension associated with diastolic heart failure (dilate-1). *Chest*. 2014;146:1274-1285
184. Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, Barst RJ, Benza RL, Liou TG, Turner M, Giles S, Feldkircher K, Miller DP, McGoon MD. Pulmonary arterial hypertension baseline characteristics from the reveal registry. *Chest*. 2010;137:376-387
 185. Ling Y, Johnson MK, Kiely DG, Condliffe R, Elliot CA, Simon J, Gibbs R, Howard LS, Pepke-Zaba J, Sheares KKK, Corriss PA, Fisher AJ, Lordan JL, Gaine S, Coghlan JG, Wort SJ, Gatzoulis MA, Peacock AJ. Changing demographics, epidemiology, and survival of incident pulmonary arterial hypertension results from the pulmonary hypertension registry of the united kingdom and ireland. *American Journal of Respiratory and Critical Care Medicine*. 2012;186:790-796
 186. Hoepfer MM, Huscher D, Ghofrani HA, Delcroix M, Distler O, Schweiger C, Grunig E, Staehler G, Rosenkranz S, Halank M, Held M, Grohe C, Lange TJ, Behr J, Klose H, Wilkens H, Filusch A, Germann M, Ewert R, Seyfarth HJ, Olsson KM, Opitz CF, Gaine SP, Vizza CD, Vonk-Noordegraaf A, Kaemmerer H, Gibbs JSR, Pittrow D. Elderly patients diagnosed with idiopathic pulmonary arterial hypertension: Results from the compera registry. *International Journal of Cardiology*. 2013;168:871-880
 187. Lam CSP, Borlaug BA, Kane GC, Enders FT, Rodeheffer RJ, Redfield MM. Age-associated increases in pulmonary artery systolic pressure in the general population. *Circulation*. 2009;119:2663-2670
 188. Pugh ME, Sivarajan L, Wang L, Robbins IM, Newman JH, Hemnes AR. Causes of pulmonary hypertension in the elderly. *Chest*. 2014;146:159-166
 189. Redfield MM, Jacobsen SJ, Burnett JC, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community - appreciating the scope of the heart failure epidemic. *Jama-Journal of the American Medical Association*. 2003;289:194-202
 190. Kitzman DW, Gardin JM, Gottdiener JS, Arnold A, Boineau R, Aurigemma G, Marino EK, Lyles M, Cushman M, Enright PL, Grp CHSR. Importance of heart failure with preserved systolic function in patients ≥ 65 years at age. *American Journal of Cardiology*. 2001;87:413-419
 191. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: Pathophysiology, diagnosis, and treatment. *European Heart Journal*. 2011;32:670-+
 192. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *New England Journal of Medicine*. 2006;355:251-259
 193. Yancy CW, Lopatin M, Stevenson LW, De Marco T, Fonarow GC, Investig ASAC. Clinical presentation, management, and in-hospital outcomes of patients admitted with acute decompensated heart failure with preserved systolic function - a report from the acute decompensated heart failure national registry (adhere) database. *Journal of the American College of Cardiology*. 2006;47:76-84
 194. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang JM, Haouzi A, Gong YY, Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. *New England Journal of Medicine*. 2006;355:260-269
 195. Thenappan T, Prins KW, Cogswell R, Shah SJ. Pulmonary hypertension secondary to heart failure with preserved ejection fraction. *Canadian Journal of Cardiology*. 2015;31:430-439
 196. Hoepfer MM, Bogaard HJ, Condliffe R, Frantz R, Khanna D, Kurzyna M, Langleben D, Manes A, Satoh T, Torres F, Wilkins MR, Badesch DB. Definitions and diagnosis of pulmonary hypertension. *Journal of the American College of Cardiology*. 2013;62:D42-D50

197. Fang JC, DeMarco T, Givertz MM, Borlaug BA, Lewis GD, Rame JE, Gomberg-Maitland M, Murali S, Frantz RP, McGlothlin D, Horn EM, Benza RL. World health organization pulmonary hypertension group 2: Pulmonary hypertension due to left heart disease in the adult-a summary statement from the pulmonary hypertension council of the international society for heart and lung transplantation. *Journal of Heart and Lung Transplantation*. 2012;31:913-933
198. Naeije R, Vachiery JL, Yerly P, Vanderpool R. The transpulmonary pressure gradient for the diagnosis of pulmonary vascular disease. *European Respiratory Journal*. 2013;41:217-223
199. Vachiery JL, Adir Y, Barbera JA, Champion H, Coghlan JG, Cottin V, De Marco T, Galie N, Ghio S, Gibbs JSR, Martinez F, Semigran M, Simonneau G, Wells A, Seeger W. Pulmonary hypertension due to left heart diseases. *Journal of the American College of Cardiology*. 2013;62:D100-D108
200. Klapholz M, Maurer M, Lowe AM, Messineo F, Meisner JS, Mitchell J, Kalman J, Phillips RA, Steingart R, Brown EJ, Berkowitz R, Moskowitz R, Soni A, Mancini D, Bijou R, Sehhat K, Varshneya N, Kukin M, Katz SD, Sleeper LA, Le Jemtel TH, New York Heart Failure C. Hospitalization for heart failure in the presence of a normal left ventricular ejection fraction - results of the new york heart failure registry. *Journal of the American College of Cardiology*. 2004;43:1432-1438
201. Leung CC, Moondra V, Catherwood E, Andrus BW. Prevalence and risk factors of pulmonary hypertension in patients with elevated pulmonary venous pressure and preserved ejection fraction. *American Journal of Cardiology*. 2010;106:284-286
202. Schwartzberg S, Redfield MM, From AM, Sorajja P, Nishimura RA, Borlaug BA. Effects of vasodilation in heart failure with preserved or reduced ejection fraction implications of distinct pathophysiologies on response to therapy. *Journal of the American College of Cardiology*. 2012;59:442-451
203. Lam CSP, Roger VL, Rodeheffer RJ, Borlaug BA, Enders FT, Redfield MM. Pulmonary hypertension in heart failure with preserved ejection fraction a community-based study. *Journal of the American College of Cardiology*. 2009;53:1119-1126
204. McQuillan BM, Picard MH, Leavitt M, Weyman AE. Clinical correlates and reference intervals for pulmonary artery systolic pressure among echocardiographically normal subjects. *Circulation*. 2001;104:2797-2802
205. Mohammed SF, Hussain I, Abou Ezzeddine OF, Takahama H, Kwon SH, Forfia P, Roger VL, Redfield MM. Right ventricular function in heart failure with preserved ejection fraction a community-based study. *Circulation*. 2014;130:2310-U2186
206. Shah AM, Shah SJ, Anand IS, Sweitzer NK, O'Meara E, Heitner JF, Sopko G, Li GC, Assmann SF, McKinlay SM, Pitt B, Pfeffer MA, Solomon SD, Investigators T. Cardiac structure and function in heart failure with preserved ejection fraction baseline findings from the echocardiographic study of the treatment of preserved cardiac function heart failure with an aldosterone antagonist trial. *Circulation-Heart Failure*. 2014;7:104-115
207. Shah AM, Claggett B, Sweitzer NK, Shah SJ, Anand IS, O'Meara E, Desai AS, Heitner JF, Li GC, Fang J, Rouleau J, Zile MR, Markov V, Ryabov V, Reis G, Assmann SF, McKinlay SM, Pitt B, Pfeffer MA, Solomon SD. Cardiac structure and function and prognosis in heart failure with preserved ejection fraction findings from the echocardiographic study of the treatment of preserved cardiac function heart failure with an aldosterone antagonist (topcat) trial. *Circulation-Heart Failure*. 2014;7:740-U116
208. Shah SJ, Katz DH, Deo RC. Phenotypic spectrum of heart failure with preserved ejection fraction. *Heart Failure Clinics*. 2014;10:407-+

209. Soufer R, Wohlgeleerter D, Vita NA, Amuchestegui M, Sostman HD, Berger HJ, Zaret BL. Intact systolic left-ventricular function in clinical congestive heart-failure. *American Journal of Cardiology*. 1985;55:1032-1036
210. Zile MR, Baicu CF, Gaasch WH. Diastolic heart failure - abnormalities in active relaxation and passive stiffness of the left ventricle. *New England Journal of Medicine*. 2004;350:1953-1959
211. Weber KT, Brilla CG, Janicki JS. Myocardial fibrosis - functional-significance and regulatory factors. *Cardiovascular Research*. 1993;27:341-348
212. Ahmed SH, Clark LL, Pennington WR, Webb CS, Bonnema DD, Leonardi AH, McClure CD, Spinale FG, Zile MR. Matrix metalloproteinases/tissue inhibitors of metalloproteinases - relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease. *Circulation*. 2006;113:2089-2096
213. van Heerebeek L, Borbely A, Niessen HWM, Bronzwaer JGF, van der Velden J, Stienen GJM, Linke WA, Laarman GJ, Paulus WJ. Myocardial structure and function differ in systolic and diastolic heart failure. *Circulation*. 2006;113:1966-1973
214. Borbely A, van der Velden J, Papp Z, Bronzwaer JGF, Edes I, Stienen GJM, Paulus WJ. Cardiomyocyte stiffness in diastolic heart failure. *Circulation*. 2005;111:774-781
215. Phan TT, Abozguia K, Shivu GN, Mahadevan G, Ahmed I, Williams L, Dwivedi G, Patel K, Steendijk P, Ashrafian H, Henning A, Frenneaux M. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. *Journal of the American College of Cardiology*. 2009;54:402-409
216. Borlaug BA, Lam CSP, Roger VL, Rodeheffer RJ, Redfield MM. Contractility and ventricular systolic stiffening in hypertensive heart disease insights into the pathogenesis of heart failure with preserved ejection fraction. *Journal of the American College of Cardiology*. 2009;54:410-418
217. Kawaguchi M, Hay I, Fetcs B, Kass DA. Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction - implications for systolic and diastolic reserve limitations. *Circulation*. 2003;107:714-720
218. Hundley WG, Kitzman DW, Morgan TM, Hamilton CA, Darty SN, Stewart KP, Herrington DM, Link KM, Little WC. Cardiac cycle-dependent changes in aortic area and distensibility are reduced in older patients with isolated diastolic heart failure and correlate with exercise intolerance. *Journal of the American College of Cardiology*. 2001;38:796-802
219. Borlaug BA, Melenovsky V, Russell SD, Kessler K, Pacak K, Becker LC, Kass DA. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation*. 2006;114:2138-2147
220. Damy T, Goode KM, Kallvikbacka-Bennett A, Lewinter C, Hobkirk J, Nikitin NP, Dubois-Rande JL, Hittinger L, Clark AL, Cleland JGF. Determinants and prognostic value of pulmonary arterial pressure in patients with chronic heart failure. *European Heart Journal*. 2010;31:2280-2290
221. Heath D, Edwards JE. Histological changes in the lung in diseases associated with pulmonary venous hypertension. *British Journal of Diseases of the Chest*. 1959;53:8-18
222. Gerges C, Gerges M, Lang MB, Zhang YH, Jakowitsch J, Probst P, Maurer G, Lang IM. Diastolic pulmonary vascular pressure gradient a predictor of prognosis in "out-of-proportion" pulmonary hypertension. *Chest*. 2013;143:758-766

223. Tatebe S, Fukumoto Y, Sugimura K, Miyamichi-Yamamoto S, Aoki T, Miura Y, Nochioka K, Satoh K, Shimokawa H. Clinical significance of reactive post-capillary pulmonary hypertension in patients with left heart disease. *Circulation Journal*. 2012;76:1235-1244
224. Redfield MM, Jacobsen SJ, Borlaug BA, Rodeheffer RJ, Kass DA. Age- and gender-related ventricular-vascular stiffening - a community-based study. *Circulation*. 2005;112:2254-2262
225. Puwanant S, Priester TC, Mookadam F, Bruce CJ, Redfield MM, Chandrasekaran K. Right ventricular function in patients with preserved and reduced ejection fraction heart failure. *European Journal of Echocardiography*. 2009;10:733-737
226. Melenovsky V, Hwang SJ, Lin G, Redfield MM, Borlaug BA. Right heart dysfunction in heart failure with preserved ejection fraction. *European Heart Journal*. 2014;35:3452-3462
227. Kjaergaard J, Akkan D, Iversen KK, Kjoller E, Kober L, Torp-Pedersen C, Hassager C. Prognostic importance of pulmonary hypertension in patients with heart failure. *American Journal of Cardiology*. 2007;99:1146-1150
228. Burke MA, Katz DH, Beussink L, Selvaraj S, Gupta DK, Fox J, Chakrabarti S, Sauer AJ, Rich JD, Freed BH, Shah SJ. Prognostic importance of pathophysiologic markers in patients with heart failure and preserved ejection fraction. *Circulation-Heart Failure*. 2014;7:288-299
229. Robbins IM, Newman JH, Johnson RF, Hemnes AR, Fremont RD, Piana RN, Zhao DX, Byrne DW. Association of the metabolic syndrome with pulmonary venous hypertension. *Chest*. 2009;136:31-36
230. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Cottin V, Degano B, Jais X, Montani D, Souza R, Simonneau G. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation*. 2010;122:156-163
231. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, Frost A, Barst RJ, Badesch DB, Elliott CG, Liou TG, McGoon MD. Predicting survival in pulmonary arterial hypertension insights from the registry to evaluate early and long-term pulmonary arterial hypertension disease management (reveal). *Circulation*. 2010;122:164-U138
232. Galie N, Corris PA, Frost A, Girgis RE, Granton J, Jing ZC, Klepetko W, McGoon MD, McLaughlin VV, Preston IR, Rubin LJ, Sandoval J, Seeger W, Keogh A. Updated treatment algorithm of pulmonary arterial hypertension. *Journal of the American College of Cardiology*. 2013;62:D60-D72
233. Fisher MR, Mathai SC, Champion HC, Girgis RE, Houston-Harris T, Hummers L, Krishnan JA, Wigley F, Hassoun PM. Clinical differences between idiopathic and scleroderma-related pulmonary hypertension. *Arthritis and Rheumatism*. 2006;54:3043-3050
234. Clements PJ, Tan M, McLaughlin VV, Oudiz RJ, Tapson VF, Channick RN, Rubin LJ, Langer A, Pulm Arterial Hypertension Q. The pulmonary arterial hypertension quality enhancement research initiative: Comparison of patients with idiopathic pah to patients with systemic sclerosis-associated pah. *Annals of the Rheumatic Diseases*. 2012;71:249-252
235. Sievers B, Kirchberg S, Addo M, Bakan A, Brandts B, Trappe HJ. Assessment of left atrial volumes in sinus rhythm and atrial fibrillation using the biplane area-length method and cardiovascular magnetic resonance imaging with truefisp. *Journal of Cardiovascular Magnetic Resonance*. 2004;6:855-863
236. Gibbs JSR. Consensus statement on the management of pulmonary hypertension in clinical practice in the uk and ireland. *Thorax*. 2008;63:1-41

237. Ryan JJ, Rich JD, Thiruvoipati T, Swamy R, Kim GH, Rich S. Current practice for determining pulmonary capillary wedge pressure predisposes to serious errors in the classification of patients with pulmonary hypertension. *American Heart Journal*. 2012;163:589-594
238. Luchsinger PC, Seipp HW, Patel DJ. Relationship of pulmonary artery-wedge pressure to left atrial pressure in man. *Circulation Research*. 1962;11:315-&
239. Halpern SD, Taichman DB. Misclassification of pulmonary hypertension due to reliance on pulmonary capillary wedge pressure rather than left ventricular end-diastolic pressure. *Chest*. 2009;136:37-43
240. Fujimoto N, Borlaug BA, Lewis GD, Hastings JL, Shafer KM, Bhella PS, Carrick-Ranson G, Levine BD. Hemodynamic responses to rapid saline loading the impact of age, sex, and heart failure. *Circulation*. 2013;127:55-+
241. Corradi D, Maestri R, Macchi E, Callegari S. The atria: From morphology to function. *Journal of Cardiovascular Electrophysiology*. 2011;22:223-235
242. Barbier P, Solomon SB, Schiller NB, Glantz SA. Left atrial relaxation and left ventricular systolic function determine left atrial reservoir function. *Circulation*. 1999;100:427-436
243. Prioli A, Marino P, Lanzoni L, Zardini P. Increasing degrees of left ventricular filling impairment modulate left atrial function in humans. *American Journal of Cardiology*. 1998;82:756-761
244. Gibson DN, Di Biase L, Mohanty P, Patel JD, Bai R, Sanchez J, Burkhardt JD, Heywood JT, Johnson AD, Rubenson DS, Horton R, Gallinghouse GJ, Beheiry S, Curtis GP, Cohen DN, Lee MY, Smith MR, Gopinath D, Lewis WR, Natale A. Stiff left atrial syndrome after catheter ablation for atrial fibrillation: Clinical characterization, prevalence, and predictors. *Heart Rhythm*. 2011;8:1364-1371
245. Thomas L, Levett K, Boyd A, Leung DY, Schiller NB, Ross DL. Compensatory changes in atrial volumes with normal aging: Is atrial enlargement inevitable? *Journal of the American College of Cardiology*. 2002;40:1630-1635
246. Boyd AC, Schiller NB, Leung D, Ross DL, Thomas L. Atrial dilation and altered function are mediated by age and diastolic function but not before the eighth decade. *Jacc-Cardiovascular Imaging*. 2011;4:234-242
247. Abhayaratna WP, Seward JB, Appleton CP, Douglas PS, Oh JK, Tajik AJ, Tsang TSM. Left atrial size - physiologic determinants and clinical applications. *Journal of the American College of Cardiology*. 2006;47:2357-2363
248. Maceira AM, Cosin-Sales J, Roughton M, Prasad SK, Pennell DJ. Reference left atrial dimensions and volumes by steady state free precession cardiovascular magnetic resonance. *Journal of Cardiovascular Magnetic Resonance*. 2010;12
249. Lester SJ, Tajik AJ, Nishimura RA, Khandheria BK, Seward JB, Oh JK. Unlocking the mysteries of diastolic function - deciphering the rosetta stone 10 years later. *Journal of the American College of Cardiology*. 2008;51:679-689
250. Tsang TSM, Barnes ME, Gersh BJ, Bailey KR, Seward JB. Left atrial volume as a morphophysiologic expression of left ventricular diastolic dysfunction and relation to cardiovascular risk burden. *American Journal of Cardiology*. 2002;90:1284-1289
251. Osranek M, Seward JB, Buschenreithner B, Bergler-Klein J, Heger M, Klaar U, Binder T, Maurer G, Zehetgruber M. Imaging and diagnostic testing - diastolic function assessment in clinical practice: The value of 2-dimensional echocardiography. *American Heart Journal*. 2007;154:130-136
252. Barberato SH, Pecoits R. Usefulness of left atrial volume for the differentiation of normal from pseudonormal diastolic function pattern in patients on hemodialysis. *Journal of the American Society of Echocardiography*. 2007;20:359-365
253. Simek CL, Feldman MD, Haber HL, Wu CC, Jayaweera AR, Kaul S. Relationship between left ventricular wall thickness and left atrial size: Comparison with other measures of diastolic function. *Journal of the American Society of*

- Echocardiography : official publication of the American Society of Echocardiography*. 1995;8:37-47
254. Tan YT, Wenzelburger F, Lee E, Nightingale P, Heatlie G, Leyva F, Sanderson JE. Reduced left atrial function on exercise in patients with heart failure and normal ejection fraction. *Heart*. 2010;96:1017-1023
 255. Kim H, Jun DW, Cho YK, Nam CW, Han SW, Hur SH, Kim YN, Kim KB. The correlation of left atrial volume index to the level of n-terminal pro-bnp in heart failure with a preserved ejection fraction. *Echocardiography-a Journal of Cardiovascular Ultrasound and Allied Techniques*. 2008;25:961-967
 256. Lester SJ, Ryan EW, Schiller NB, Foster E. Best method in clinical practice and in research studies to determine left atrial size. *American Journal of Cardiology*. 1999;84:829-832
 257. Jarvinen V, Kupari M, Hekali P, Poutanen VP. Assessment of left atrial volumes and phasic function using cine magnetic-resonance-imaging in normal subjects. *American Journal of Cardiology*. 1994;73:1135-1138
 258. Whitlock M, Garg A, Gelow J, Jacobson T, Broberg C. Comparison of left and right atrial volume by echocardiography versus cardiac magnetic resonance imaging using the area-length method. *American Journal of Cardiology*. 2010;106:1345-1350
 259. Hof IE, Velthuis BK, Van Driel VJ, Wittkampfh FH, Hauer RN, Loh P. Left atrial volume and function assessment by magnetic resonance imaging. *Journal of Cardiovascular Electrophysiology*. 2010;21:1247-1250
 260. Zareian M, Ciuffo L, Habibi M, Opdahl A, Chamera EH, Wu CO, Bluemke DA, Lima JAC, Venkatesh BA. Left atrial structure and functional quantitation using cardiovascular magnetic resonance and multimodality tissue tracking: Validation and reproducibility assessment. *Journal of Cardiovascular Magnetic Resonance*. 2015;17:52-52
 261. Takagi Y, Ehara S, Okuyama T, Shirai N, Yamashita H, Sugioka K, Kitamura H, Ujino K, Hozumi T, Yoshiyama M. Comparison of determinations of left atrial volume by the biplane area-length and simpson's methods using 64-slice computed tomography. *Journal of Cardiology*. 2009;53:257-264
 262. Nacif MS, Barranhas AD, Turkbey E, Marchiori E, Kawel N, Mello RAF, Falcao RO, Oliveira AC, Rochitte CE. Left atrial volume quantification using cardiac mri in atrial fibrillation: Comparison of the simpson's method with biplane area-length, ellipse, and three-dimensional methods. *Diagnostic and Interventional Radiology*. 2013;19:213-220
 263. Jiamsripong P, Honda T, Reuss CS, Hurst RT, Chaliki HP, Grill DE, Schneck SL, Tyler R, Khandheria BK, Lester SJ. Three methods for evaluation of left atrial volume. *European Journal of Echocardiography*. 2008;9:351-355
 264. Agner BFR, Kuhl JT, Linde JJ, Kofoed KF, Akeson P, Rasmussen BV, Jensen GB, Dixen U. Assessment of left atrial volume and function in patients with permanent atrial fibrillation: Comparison of cardiac magnetic resonance imaging, 320-slice multi-detector computed tomography, and transthoracic echocardiography. *European Heart Journal-Cardiovascular Imaging*. 2014;15:532-540
 265. Rabbat MG, Wilber D, Thomas K, Malick O, Bashir A, Agrawal A, Biswas S, Sanagala T, Syed MA. Left atrial volume assessment in atrial fibrillation using multimodality imaging: A comparison of echocardiography, invasive three-dimensional carto and cardiac magnetic resonance imaging. *International Journal of Cardiovascular Imaging*. 2015;31:1011-1018
 266. Hudsmith LE, Cheng ASH, Tyler DJ, Shirodaria C, Lee J, Petersen SE, Francis JM, Clarke K, Robson MD, Neubauer S. Assessment of left atrial volumes at 1.5 tesla and 3 tesla using flash and ssfp cine imaging. *Journal of Cardiovascular Magnetic Resonance*. 2007;9:673-679

267. Sievers B, Addo M, Kirchberg S, Bakan A, Jofin-Puthenveetil B, Franken U, Trappe HJ. How much are atrial volumes and ejection fractions assessed by cardiac magnetic resonance imaging influenced by the ecg gating method? *Journal of Cardiovascular Magnetic Resonance*. 2005;7:587-593
268. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the american society of echocardiography and the european association of cardiovascular imaging. *European Heart Journal-Cardiovascular Imaging*. 2015;16:233-271
269. Peacock AJ, Murphy NF, McMurray JJV, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. *European Respiratory Journal*. 2007;30:104-109
270. Crawley SF, Johnson MK, Peacock AJ. The changing face of pulmonary hypertension: The role of heart and lung disease. *Thorax*. 2010;65:A89-A89
271. Barst RJ, Rubin LJ, Long WA, McGoon MD, Rich S, Badesch DB, Groves BM, Tapson VF, Bourge RC, Brundage BH, Koerner SK, Langleben D, Keller CA, Murali S, Uretsky BF, Clayton LM, Jobsis MM, Blackburn SD, Shortino D, Crow JW. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. *New England Journal of Medicine*. 1996;334:296-301
272. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G, Grp SS. Sildenafil citrate therapy for pulmonary arterial hypertension. *New England Journal of Medicine*. 2005;353:2148-2157
273. Bonderman D, Wexberg P, Martischnig AM, Heinzl H, Lang MB, Sadushi R, Skoro-Sajer N, Lang IM. A noninvasive algorithm to exclude pre-capillary pulmonary hypertension. *European Respiratory Journal*. 2011;37:1096-1103
274. Caselli S, Canali E, Foschi ML, Santini D, Di Angelantonio E, Pandian NG, De Castro S. Long-term prognostic significance of three-dimensional echocardiographic parameters of the left ventricle and left atrium. *European Journal of Echocardiography*. 2010;11:250-256
275. Kizer JR, Bella JN, Palmieri V, Liu JE, Best LG, Lee ET, Roman MJ, Devereux RB. Left atrial diameter as an independent predictor of first clinical cardiovascular events in middle-aged and elderly adults: The strong heart study (shs). *American Heart Journal*. 2006;151:412-418
276. Tsang TSM, Abhayaratna WP, Barnes ME, Miyasaka Y, Gersh BJ, Bailey KR, Cha SS, Seward JB. Prediction of cardiovascular outcomes with left atrial size - is volume superior to area or diameter? *Journal of the American College of Cardiology*. 2006;47:1018-1023
277. Patel RK, Jardine AGM, Mark PB, Cunningham AF, Steedman T, Powell JR, McQuarrie EP, Stevens KK, Dargie HJ, Jardine AG. Association of left atrial volume with mortality among esrd patients with left ventricular hypertrophy referred for kidney transplantation. *American Journal of Kidney Diseases*. 2010;55:1088-1096
278. Hee L, Nguyen T, Whatmough M, Descallar J, Chen J, Kapila S, French JK, Thomas L. Left atrial volume and adverse cardiovascular outcomes in unselected patients with and without ckd. *Clinical Journal of the American Society of Nephrology*. 2014;9:1369-1376
279. Habibi M, Chahal H, Opdahl A, Gjesdal O, Helle-Valle TM, Heckbert SR, McClelland R, Wu CL, Shea S, Hundley G, Bluemke DA, Lima JAC. Association

- of cmr-measured la function with heart failure development results from the mesa study results from the mesa study. *Jacc-Cardiovascular Imaging*. 2014;7:570-579
280. Ristow B, Ali S, Whooley MA, Schiller NB. Usefulness of left atrial volume index to predict heart failure hospitalization and mortality in ambulatory patients with coronary heart disease and comparison to left ventricular ejection fraction (from the heart and soul study). *American Journal of Cardiology*. 2008;102:70-76
 281. Montefusco A, Biasco L, Blandino A, Cristoforetti Y, Scaglione M, Caponi D, Di Donna P, Boffano C, Cesarani F, Coin D, Perversi J, Gaita F. Left atrial volume at mri is the main determinant of outcome after pulmonary vein isolation plus linear lesion ablation for paroxysmal-persistent atrial fibrillation. *Journal of Cardiovascular Medicine*. 2010;11:593-598
 282. Rodevand O, Bjornerheim R, Ljosland M, Maehle J, Smith HJ, Ihlen H. Left atrial volumes assessed by three- and two-dimensional echocardiography compared to mri estimates. *International Journal of Cardiac Imaging*. 1999;15:397-410
 283. Galie N, Barbera JA, Frost AE, Ghofrani HA, Hoeper MM, McLaughlin VV, Peacock AJ, Simonneau G, Vachiery JL, Grunig E, Oudiz RJ, Vonk-Noordegraaf A, White RJ, Blair C, Gillies H, Miller KL, Harris JHN, Langley J, Rubin LJ, Ambition I. Initial use of ambrisentan plus tadalafil in pulmonary arterial hypertension. *New England Journal of Medicine*. 2015;373:834-844
 284. Riedel M, Stanek V, Widimsky J, Prerovsky I. Longterm follow-up of patients with pulmonary thromboembolism - late prognosis and evolution of hemodynamic and respiratory data. *Chest*. 1982;81:151-158
 285. Han MK, McLaughlin VV, Criner GJ, Martinez FJ. Pulmonary diseases and the heart. *Circulation*. 2007;116:2992-3005
 286. Voelkel NF, Gomez-Arroyo J, Abbate A, Bogaard HJ, Nicolls MR. Pathobiology of pulmonary arterial hypertension and right ventricular failure. *European Respiratory Journal*. 2012;40:1555-1565
 287. Simonneau G, Rubin LJ, Galie N, Barst RJ, Fleming TR, Frost AE, Engel PJ, Kramer MR, Burgess G, Collings L, Cossons N, Sitbon O, Badesch DB, Grp PS. Addition of sildenafil to long-term intravenous epoprostenol therapy in patients with pulmonary arterial hypertension a randomized trial. *Ann. Intern. Med*. 2008;149:521-W102
 288. Gabler NB, French B, Strom BL, Palevsky HI, Taichman DB, Kawut SM, Halpern SD. Validation of 6-minute walk distance as a surrogate end point in pulmonary arterial hypertension trials. *Circulation*. 2012;126:349-+
 289. Rubin LJ, Mendoza J, Hood M, McGoon M, Barst R, Williams WB, Diehl JH, Crow J, Long W. Treatment of primary pulmonary-hypertension with continuous intravenous prostacyclin (epoprostenol) - results of a randomized trial. *Ann. Intern. Med*. 1990;112:485-491
 290. Olschewski H, Simonneau G, Galie N, Higenbottam T, Naeije R, Rubin LJ, Nikkho S, Speich R, Hoeper MM, Behr J, Winkler J, Sitbon O, Popov W, Ghofrani HA, Manes A, Kiely DG, Ewert R, Siedentop H, Seeger W. Inhaled iloprost for severe pulmonary hypertension. *New England Journal of Medicine*. 2002;347:322-329
 291. Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M, Simonneau G, Bosentan Randomized Trial E. Bosentan therapy for pulmonary arterial hypertension. *New England Journal of Medicine*. 2002;346:896-903
 292. Humbert M, Barst RJ, Robbins IM, Channick RN, Galie N, Boonstra A, Rubin LJ, Horn EM, Manes A, Simonneau G. Combination of bosentan with epoprostenol in pulmonary arterial hypertension: Breathe-2. *European Respiratory Journal*. 2004;24:353-359
 293. Wilkins MR, Paul GA, Strange JW, Tunariu N, Gin-Sing W, Banya WA, Westwood MA, Stefanidis A, Ng LL, Pennell DJ, Mohiaddin RH,

- Nihoyannopoulos P, Gibbs JSR. Sildenafil versus endothelin receptor antagonist for pulmonary hypertension (seraph) study. *American Journal of Respiratory and Critical Care Medicine*. 2005;171:1292-1297
294. McLaughlin VV, Oueliz RJ, Frost A, Tapson VF, Murali S, Channick RN, Badesch DB, Barst RJ, Hsu HH, Rubin LJ. Randomized study of adding inhaled iloprost to existing bosentan in pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2006;174:1257-1263
295. Hoepfer MM, Leuchte H, Halank M, Wilkens H, Meyer FJ, Seyfarth HJ, Wensel R, Ripken F, Bremer H, Kluge S, Hoeffken G, Behr J. Combining inhaled iloprost with bosentan in patients with idiopathic pulmonary arterial hypertension. *European Respiratory Journal*. 2006;28:691-694
296. Galie N, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, Gerber MJ, Dufton C, Wiens BL, Rubin LJ, Ambrisentan Pulm Arterial H. Ambrisentan for the treatment of pulmonary arterial hypertension - results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (aries) study 1 and 2. *Circulation*. 2008;117:3010-3019
297. Galie N, Manes A, Negro L, Palazzini M, Bacchi-Reggiani ML, Branzi A. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. *European Heart Journal*. 2009;30:394-403
298. Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the reveal registry. *Chest*. 2012;142:448-456
299. Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JSR, Vrapic F, Das C, Elliot CA, Johnson M, DeSoyza J, Torpy C, Goldsmith K, Hodgkins D, Hughes RJ, Pepke-Zaba J, Coghlan JG. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. *American Journal of Respiratory and Critical Care Medicine*. 2009;179:151-157
300. Koh ET, Lee P, Gladman DD, AbuShakra M. Pulmonary hypertension in systemic sclerosis: An analysis of 17 patients. *British Journal of Rheumatology*. 1996;35:989-993
301. Barst RJ, McGoon M, McLaughlin V, Tapson V, Oudiz R, Shapiro S, Robbins IM, Channick R, Badesch D, Rayburn BK, Flinchbaugh R, Sigman J, Arneson C, Jeffs R, Beraprost Study G. Beraprost therapy for pulmonary arterial hypertension. *Journal of the American College of Cardiology*. 2003;41:2119-2125
302. Blalock SE, Matulevicius S, Mitchell LC, Reimold S, Warner J, Peshock R, Torres F, Chin KM. Long-term outcomes with ambrisentan monotherapy in pulmonary arterial hypertension. *Journal of Cardiac Failure*. 2010;16:121-127
303. van Wolferen SA, Boonstra A, Marcus JT, Marques KMJ, Bronzwaer JGF, Postmus PE, Vonk-Noordegraaf A. Right ventricular reverse remodelling after sildenafil in pulmonary arterial hypertension. *Heart*. 2006;92:1860-1861
304. Ruiz MJ, Escribano P, Delgado JF, Jimenez C, Tello R, Gomez MA, de la Calzada CS. Efficacy of sildenafil as a rescue therapy for patients with severe pulmonary arterial hypertension and given long-term treatment with prostanoids: 2-year experience. *Journal of Heart and Lung Transplantation*. 2006;25:1353-1357
305. Lee W-TN, Brown A, Peacock AJ, Johnson MK. Use of non-invasive haemodynamic measurements to detect treatment response in precapillary pulmonary hypertension. *Thorax*. 2011;66:810-814
306. Chin KM, Kingman M, de Lemos JA, Warner JJ, Reimold S, Peshock R, Torres F. Changes in right ventricular structure and function assessed using cardiac magnetic resonance imaging in bosentan-treated patients with pulmonary arterial hypertension. *American Journal of Cardiology*. 2008;101:1669-1672

307. van de Veerdonk MC, Kind T, Marcus JT, Mauritz G-J, Heymans MW, Bogaard H-J, Boonstra A, Marques KMJ, Westerhof N, Vonk-Noordegraaf A. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. *Journal of the American College of Cardiology*. 2011;58:2511-2519
308. Mukerjee D, St George D, Coleiro B, Knight C, Denton CP, Davar J, Black CM, Coghlan JG. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension: Application of a registry approach. *Annals of the Rheumatic Diseases*. 2003;62:1088-1093
309. Avouac J, Airo P, Meune C, Beretta L, Dieude P, Caramaschi P, Tiev K, Cappelli S, Diot E, Vacca A, Cracowski JL, Sibilia J, Kahan A, Matucci-Cerinic M, Allanore Y. Prevalence of pulmonary hypertension in systemic sclerosis in european caucasians and metaanalysis of 5 studies. *Journal of Rheumatology*. 2010;37:2290-2298
310. Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. *Annals of the Rheumatic Diseases*. 2007;66:940-944
311. Chaisson NF, Hassoun PM. Systemic sclerosis-associated pulmonary arterial hypertension. *Chest*. 2013;144:1346-1356
312. Hsu VM, Chung L, Hummers LK, Wigley F, Simms R, Bolster M, Silver R, Fischer A, Hinchcliff ME, Varga J, Goldberg AZ, Derk CT, Schioppa E, Khanna D, Shapiro LS, Domsic RT, Medsger T, Mayes MD, Furst D, Csuka ME, Molitor JA, Alkassab F, Steen VD. Development of pulmonary hypertension in a high-risk population with systemic sclerosis in the pulmonary hypertension assessment and recognition of outcomes in scleroderma (pharos) cohort study. *Seminars in Arthritis and Rheumatism*. 2014;44:55-62
313. Hinchcliff M, Fischer A, Schioppa E, Steen VD. Pulmonary hypertension assessment and recognition of outcomes in scleroderma (pharos): Baseline characteristics and description of study population. *Journal of Rheumatology*. 2011;38:2172-2179
314. Randone SB, Guiducci S, Cerinic MM. Musculoskeletal involvement in systemic sclerosis. *Best Practice & Research in Clinical Rheumatology*. 2008;22:339-350
315. Impens AJ, Wangkaew S, Seibold JR. The 6-minute walk test in scleroderma how measuring everything measures nothing. *Rheumatology*. 2008;47:V68-V69
316. Kherbeck N, Tamby MC, Bussone G, Dib H, Perros F, Humbert M, Mouthon L. The role of inflammation and autoimmunity in the pathophysiology of pulmonary arterial hypertension. *Clinical Reviews in Allergy & Immunology*. 2013;44:31-38
317. Dorfmueller P, Humbert M, Perros F, Sanchez O, Simonneau G, Muller KM, Capron F. Fibrous remodeling of the pulmonary venous system in pulmonary arterial hypertension associated with connective tissue diseases. *Human Pathology*. 2007;38:893-902
318. Gabrielli A, Avvedimento EV, Krieg T. Mechanisms of disease: Scleroderma. *New England Journal of Medicine*. 2009;360:1989-2003
319. Nicolls MR, Taraseviciene-Stewart L, Rai PR, Badesch DB, Voelkel NF. Autoimmunity and pulmonary hypertension: A perspective. *European Respiratory Journal*. 2005;26:1110-1118
320. Riemekasten G, Philippe A, Nather M, Slowinski T, Muller DN, Heidecke H, Matucci-Cerinic M, Czirjak L, Lukitsch I, Becker M, Kill A, van Laar JM, Catar R, Luft FC, Burmester GR, Hegner B, Dragun D. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Annals of the Rheumatic Diseases*. 2011;70:530-536
321. Negi VS, Tripathy NK, Misra R, Nityanand S. Antiendothelial cell antibodies in scleroderma correlate with severe digital ischemia and pulmonary arterial hypertension. *Journal of Rheumatology*. 1998;25:462-466

322. Tamby MC, Humbert M, Guilpain P, Servettaz A, Dupin N, Christner JJ, Simonneau G, Fermanian J, Weill B, Guillevin L, Mouthon L. Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. *European Respiratory Journal*. 2006;28:799-807
323. Boueiz A, Mathai SC, Hummers LK, Hassoun PM. Cardiac complications of systemic sclerosis: Recent progress in diagnosis. *Current Opinion in Rheumatology*. 2010;22:696-703
324. Sadeghi S, Granton JT, Akhavan P, Pasarikovski CR, Roos AM, Thenganatt J, Moric J, Johnson SR. Survival in rheumatoid arthritis-associated pulmonary arterial hypertension compared with idiopathic pulmonary arterial hypertension. *Respirology*. 2015;20:481-487
325. Chung L, Farber HW, Benza R, Miller DP, Parsons L, Hassoun PM, McGoon M, Nicolls MR, Zamanian RT. Unique predictors of mortality in patients with pulmonary arterial hypertension associated with systemic sclerosis in the reveal registry. *Chest*. 2014;146:1494-1504
326. Lefevre G, Dauchet L, Hachulla E, Montani D, Sobanski V, Lambert M, Hatron PY, Humbert M, Launay D. Survival and prognostic factors in systemic sclerosis-associated pulmonary hypertension: A systematic review and meta-analysis. *Arthritis and Rheumatism*. 2013;65:2412-2423
327. Campo A, Mathai SC, Le Pavec J, Zaiman AL, Hummers LK, Boyce D, Houston T, Champion HC, Lechtzin N, Wigley FM, Girgis RE, Hassoun PM. Hemodynamic predictors of survival in scleroderma-related pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*. 2010;182:252-260
328. Overbeek MJ, Lankhaar JW, Westerhof N, Voskuyl AE, Boonstra A, Bronzwaer JGF, Marques KMJ, Smit EF, Dijkmans BAC, Vonk-Noordegraaf A. Right ventricular contractility in systemic sclerosis-associated and idiopathic pulmonary arterial hypertension. *European Respiratory Journal*. 2008;31:1160-1166
329. Tedford RJ, Mudd JO, Girgis RE, Mathai SC, Zaiman AL, Houston-Harris T, Boyce D, Kelemen BW, Bacher AC, Shah AA, Hummers LK, Wigley FM, Russell SD, Saggat R, Saggat R, Maughan WL, Hassoun PM, Kass DA. Right ventricular dysfunction in systemic sclerosis-associated pulmonary arterial hypertension. *Circulation. Heart failure*. 2013;6:953-963
330. Kelemen BW, Mathai SC, Tedford RJ, Damico RL, Corona-Villalobos C, Kolb TM, Chaisson NF, Harris TH, Zimmerman SL, Kamel IR, Kass DA, Hassoun PM. Right ventricular remodeling in idiopathic and scleroderma-associated pulmonary arterial hypertension: Two distinct phenotypes. *Pulmonary circulation*. 2015;5:327-334
331. Peacock AJ, Crawley S, McLure L, Blyth K, Vizza CD, Poscia R, Francone M, Iacucci I, Olschewski H, Kovacs G, Noordegraaf AV, Marcus JT, van de Veerdonk MC, Oosterveer FPT. Changes in right ventricular function measured by cardiac magnetic resonance imaging in patients receiving pulmonary arterial hypertension-targeted therapy the euro-mr study. *Circulation-Cardiovascular Imaging*. 2014;7:107-114
332. Saleh S, Liakopoulos OJ, Buckberg GD. The septal motor of biventricular function. *European Journal of Cardio-Thoracic Surgery*. 2006;29:S126-S138
333. Santamore WP, Dell'Italia LJ. Ventricular interdependence: Significant left ventricular contributions to right ventricular systolic function. *Progress in Cardiovascular Diseases*. 1998;40:289-308
334. Haeck MLA, Hoke U, Marsan NA, Holman ER, Wolterbeek R, Bax JJ, Schalij MJ, Vliegen HW, Delgado V. Impact of right ventricular dyssynchrony on left ventricular performance in patients with pulmonary hypertension. *International Journal of Cardiovascular Imaging*. 2014;30:713-720

335. Shehata ML, Harouni AA, Skrok J, Basha TA, Boyce D, Lechtzin N, Mathai SC, Girgis R, Osman NF, Lima JAC, Bluemke DA, Hassoun PM, Vogel-Claussen J. Regional and global biventricular function in pulmonary arterial hypertension: A cardiac mr imaging study. *Radiology*. 2013;266:114-122
336. Vonk Noordegraaf A, Marcus JT, Roseboom B, Postmus PE, Faes TJ, De Vries PM. The effect of right ventricular hypertrophy on left ventricular ejection fraction in pulmonary emphysema. *Chest*. 1997;112:640-645
337. Hardegree EL, Sachdev A, Fenstad ER, Villarraga HR, Frantz RP, McGoon MD, Oh JK, Ammash NM, Connolly HM, Eidem BW, Pellikka PA, Kane GC. Impaired left ventricular mechanics in pulmonary arterial hypertension identification of a cohort at high risk. *Circulation-Heart Failure*. 2013;6:748-755
338. de Groote P, Gressin V, Hachulla E, Carpentier P, Guillevin L, Kahan A, Cabane J, Frances C, Lamblin N, Diot E, Patat F, Sibilia J, Petit H, Cracowski JL, Clerson P, Humbert M, Itiner AIRSI. Evaluation of cardiac abnormalities by doppler echocardiography in a large nationwide multicentric cohort of patients with systemic sclerosis. *Annals of the Rheumatic Diseases*. 2008;67:31-36
339. Hachulla AL, Launay D, Gaxotte V, de Groote P, Lamblin N, Devos P, Hatron PY, Beregi JP, Hachulla E. Cardiac magnetic resonance imaging in systemic sclerosis: A cross-sectional observational study of 52 patients. *Annals of the Rheumatic Diseases*. 2009;68:1878-1884
340. Faludi R, Kolto G, Bartos B, Csima G, Czirjak L, Komocsi A. Five-year follow-up of left ventricular diastolic function in systemic sclerosis patients: Determinants of mortality and disease progression. *Seminars in Arthritis and Rheumatism*. 2014;44:220-227
341. Hinchcliff M, Desai CS, Varga J, Shah SJ. Prevalence, prognosis, and factors associated with left ventricular diastolic dysfunction in systemic sclerosis. *Clinical and Experimental Rheumatology*. 2012;30:S30-S37
342. Poanta L, Dadu R, Tiboc C, Rednic S, Dumitrascu D. Systolic and diastolic function in patients with systemic sclerosis. *European Journal of Internal Medicine*. 2009;20:378-382
343. Follansbee WP, Miller TR, Curtiss EI, Orié JE, Bernstein RL, Kiernan JM, Medsger TA. A controlled clinicopathological study of myocardial fibrosis in systemic-sclerosis (scleroderma). *Journal of Rheumatology*. 1990;17:656-662
344. Fox BD, Shimony A, Langleben D, Hirsch A, Rudski L, Schlesinger R, Eisenberg MJ, Joyal D, Hudson M, Boutet K, Serban A, Masetto A, Baron M. High prevalence of occult left heart disease in scleroderma-pulmonary hypertension. *European Respiratory Journal*. 2013;42:1083-1091
345. Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, Borbely A, Edes I, Handoko ML, Heymans S, Pezzali N, Pieske B, Dickstein K, Fraser AG, Brutsaert DL. How to diagnose diastolic heart failure: A consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the heart failure and echocardiography associations of the european society of cardiology. *European Heart Journal*. 2007;28:2539-2550
346. Mavrogeni S, Sfrikakis PP, Karabela G, Stavropoulos E, Spiliotis G, Gialafos E, Panopoulos S, Bournia V, Manolopoulou D, Kolovou G, Kitis G. Cardiovascular magnetic resonance imaging in asymptomatic patients with connective tissue disease and recent onset left bundle branch block. *International Journal of Cardiology*. 2014;171:82-87
347. Knight DS, Steeden JA, Moledina S, Jones A, Coghlan JG, Muthurangu V. Left ventricular diastolic dysfunction in pulmonary hypertension predicts functional capacity and clinical worsening: A tissue phase mapping study. *Journal of Cardiovascular Magnetic Resonance*. 2015;17:11

