NON-INVASIVE OUTCOME MEASURES IN PULMONARY HYPERTENSION

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AT	anaerobic threshold
AUC	area under the curve
BNP	brain natriuretic peptide
BS	Brier score
C_aO_2	oxygen content in arterial blood
$C_{a-v}O_2$	arterio-venous oxygen content difference
CCB	calcium channel blocker
C_cO_2	oxygen content in pulmonary end-capillary blood
CHD	congenital heart disease
CHF	chronic heart failure
CI	cardiac index
CO	cardiac output
COPD	chronic obstructive pulmonary disease
CoV	coefficient of variation
CPET	cardiopulmonary exercise testing
CTDPAH	connective tissue disease associated pulmonary arterial hypertension
CTEPH	chronic thromboembolic pulmonary hypertension
DLco	diffusing capacity for carbon monoxide
ECG	electrocardiography
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
HR	heart rate
IGR	inert gas rebreathing
IPAH	idiopathic pulmonary arterial hypertension
IVS	interventricular septum
LV	left ventricle
mPAP	mean pulmonary artery pressure
MRI	magnetic resonance imaging
NIH	National Institutes of Health
NTproBNP	N-terminal pro- brain natriuretic peptide
PAH	pulmonary arterial hypertension
$P_{A-a}O_2$	alveolar-arterial oxygen partial pressure gradient

P_aCO_2	arterial carbon dioxide partial pressure
PBF	pulmonary blood flow
PBF_{er}	pulmonary blood flow measured at erect rest
PBF_{ex}	pulmonary blood flow measured at exercise
PBF_{sr}	pulmonary blood flow measured at supine rest
PCWP	pulmonary capillary wedge pressure
$P_{ET}O_2$	end-tidal oxygen partial pressure
$P_{ET}CO_2$	end-tidal CO_2 partial pressure
$P_{ET}CO_{2\ nadir}$	nadir of end-tidal CO_2 partial pressure
PFO	patent foramen ovale
PH	pulmonary hypertension
PHC	Pulmonary Hypertension Connection
PoPH	portopulmonary hypertension
PVR	pulmonary vascular resistance
RAP	right atrial pressure
RER	respiratory exchange ratio
REVEAL	Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management
RHC	right heart catheterisation
ROC	receiver operating characteristics curve
RR	respiratory rate
RV	right ventricle
S_aO_2	arterial oxygen saturation
SBP	systolic blood pressure
S_cO_2	pulmonary end-capillary oxygen saturation
SD	standard deviation
SPVU	Scottish Pulmonary Vascular Unit
SSc	systemic sclerosis
SV	stroke volume
SV_{er}	stroke volume measured as erect rest
SV_{ex}	stroke volume measured at exercise
SV_{sr}	stroke volume measured at supine rest
S_VO_2	mixed venous saturation
TD	thermodilution
TLC	total lung capacity

TTCW	time to clinical worsening
QOL	quality of life
UK	United Kingdom
VCO_2	carbon dioxide output
V_D	dead space
V_D/V_T	dead space to tidal volume ratio
V_E	minute ventilation
V_E/VCO_2	ventilatory equivalent for carbon dioxide
V_E/VO_2	ventilatory equivalent for oxygen
VO_2	oxygen uptake
VO_2/HR	oxygen pulse
V/Q	ventilation to perfusion ratio
WHO FC	World Health Organisation functional class
WR_{max}	maximal work rate
6MWD	six-minute walk distance
6MWT	six-minute walk test
HR/VO_2 slope	slope of heart rate/oxygen uptake relationship
VO_2/WR slope	slope of oxygen uptake/work rate relationship
V_E/VCO_2 slope	slope of minute ventilation/carbon dioxide output relationship
% predicted 6MWD	percent predicted 6MWD
r	Pearson correlation coefficient
ρ	Spearman correlation coefficient
Δ	change
n	number

LIST OF PUBLICATIONS

Scientific papers

1. Lee WT, Ling Y, Sheares K, Pepke-Zaba J, Peacock AJ, Johnson MK. Predicting survival in pulmonary arterial hypertension in the United Kingdom. *Eur Respir J* 2012;40(3):604-611.

2. Lee WT, Brown A, Peacock AJ, Johnson MK. Use of non-invasive haemodynamic measurements to detect treatment response in precapillary pulmonary hypertension. *Thorax* 2011;66(9):810-814.

3. Lee WT, Peacock AJ, Johnson MK. The role of percent predicted six-minute walk distance in pulmonary arterial hypertension. *Eur Respir J* 2010;36(6):1294-1301.

Abstracts

1. Lee WT, Ling Y, Sheares K, Pepke-Zaba J, Peacock AJ, Johnson MK. Predicting survival in pulmonary hypertension in the United Kingdom: comparison of prognostic equations. European Respiratory Society Annual Congress 2011:4915 (oral presentation).

2. Lee WT, Brown A, Peacock AJ, Johnson MK. The use of non-invasive stroke volume measurement to assess treatment response in pulmonary arterial hypertension. European Respiratory Society Annual Congress 2010:2627 (poster presentation).

3. Lee WT, Peacock AJ, Johnson MK. A multidimensional composite score using non-invasive baseline variables to predict mortality in patients with pulmonary arterial hypertension. *Thorax* 2009;64(suppl IV):A86 (poster presentation).

4. Lee WT, Peacock AJ, Johnson MK. The prognostic significance of percent predicted six-minute walk distance in pulmonary arterial hypertension. European Respiratory Society Annual Congress 2009:166 (oral presentation). *Awarded the "Francois Brenot Award" for the best abstract in Pulmonary Vascular Science*

5. Lee WT, Brown A, Peacock AJ, Johnson MK. The effect of posture and submaximal upright exercise on stroke volume in patients with pulmonary hypertension. *Am J Respir Crit Care Med* 2009;179:A4133 (poster presentation).

6. Lee WT, Peacock AJ, Johnson MK. Stroke volume response to upright exercise in pulmonary hypertension. *Thorax* 2008;63(suppl VII):S124 (oral presentation).

7. Lee WT, Brown A, Raeside D, Peacock AJ, Johnson MK. Prognostic value of end-tidal partial pressure of carbon dioxide during cardiopulmonary exercise test in pulmonary hypertension. European Respiratory Society Annual Congress 2008:P1012 (poster presentation).

SUMMARY

Pulmonary hypertension (PH), a disease state affecting the pulmonary circulation, was first recognised in the 1950s. Obliteration of pulmonary capillary beds and vasoconstriction lead to elevated pulmonary vascular resistance (PVR) and increased right ventricular afterload. The direct consequence is impaired cardiac output (CO) response to exercise, resulting in progressive exercise limitation, and ultimately premature death from right heart failure. Despite the considerable expansion in pulmonary vasodilatory therapy in recent years, PH remains an incurable disease associated with high morbidity and mortality.

Exercise CO is an important outcome measure in PH as it is directly linked to the consequences of disease. Cardiac output is conventionally measured at right heart catheterisation (RHC). The invasive nature of this procedure does not permit serial measurements to be made readily during follow-up to assess disease progression or treatment response. As a result, six-minute walk distance (6MWD), a simple measure of submaximal exercise capacity, has been used as a surrogate of exercise CO and the primary end-point in most randomised controlled trials of pulmonary vasodilatory agents to date. However, there are recognised limitations to the ability of 6MWD to predict outcome, and this necessitates the development of alternative outcome measures which are non-invasive, reproducible and responsive to change. Measurement of CO using the inert gas rebreathing method (IGR) may be such an alternative to 6MWD. It is a direct measure of right heart function and hence disease-specific. It can be combined with submaximal constant-load exercise to provide an objective assessment independent of patient effort. This form of exercise would also allow isotime comparison of metabolic variables which were shown to be more sensitive than variables measured at peak exercise in demonstrating improved exercise capacity from therapeutic interventions in chronic obstructive pulmonary disease (COPD). Another potential alternative outcome measure is end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$). It is a marker of ventilatory inefficiency and was shown to correlate with disease severity in PH.

Accurate prognostication is central to PH management as it would inform treatment planning and patient counselling. Different strategies could be adopted to optimise the performance of existing prognostic factors. The predictive value of 6MWD may be improved by using % predicted 6MWD which adjusts for age, gender and anthropometric factors, and hence would give a more accurate representation of disease severity. A composite scoring system, combining key prognostic variables, would be more discriminatory than individual variables in predicting survival. Such prognostic equations have been derived from contemporary PH cohorts in France and the United States. Validation data published so far support their predictive value, but these equations may not perform as well in the United Kingdom (UK) as a locally derived risk score, due to differences in patient demographics and healthcare systems.

The aims of this thesis were to investigate the use of novel non-invasive exercise variables and prognostic algorithms as outcome measures in PH.

1. The first two studies evaluate the ability of IGR haemodynamic measurements and isotime metabolic variables during submaximal constant-load exercise, and $P_{ET}CO_2$ during the six-minute walk test (6MWT) to predict treatment response.

2. The last two studies explore the prognostic value of % predicted 6MWD and a novel UK-based composite risk score.

The reproducibility and clinical correlates of IGR pulmonary blood flow (PBF) and stroke volume (SV) were determined. Changes in IGR PBF and SV and isotime metabolic variables, at rest and during submaximal constant-load exercise, were assessed after three months of new or modified disease-targeted therapy in patients with precapillary PH. IGR measurements were found to have good intersession reproducibility and correlate with conventional outcome measures including World Health Organisation functional class (WHO FC), 6MWD, N-terminal pro-brain natriuretic peptide (NT-proBNP) and Cambridge Pulmonary Hypertension Outcome Review (CAMHPOR) score. Resting and submaximal exercise IGR PBF and SV were able to detect treatment response, and may be

more sensitive than 6MWD in detecting the effects of therapy in fitter patients. In comparison, isotime metabolic variables were less useful in detecting a treatment effect.

The metabolic response during the 6MWT was determined and changes in $P_{ET}CO_2$ were assessed after 3 months of new or modified disease-targeted therapy. Therapy-induced changes in the nadir of $P_{ET}CO_2$ ($P_{ET}CO_{2\ nadir}$) correlated with changes in 6MWD, but resting, end-of-walk or $P_{ET}CO_{2\ nadir}$ did not improve significantly at follow-up. Post-hoc analysis demonstrated that the study was under-powered to detect a change in $P_{ET}CO_2$ with therapy.

The prognostic performance of % predicted 6MWD, calculated using four different published reference equations, was compared with that of absolute 6MWD, at baseline and on treatment. Despite adjusting for physiological inter-subject variance, % predicted 6MWD is not superior to absolute 6MWD in predicting all-cause mortality. This may be related to limitations of existing reference equations or the use of all-cause rather than disease-specific mortality as the end-point.

Baseline mortality predictors were identified from a Scottish cohort of incident and treatment-naive PH patients, and used to derive a simple scoring system for survival prediction over time. When validated in an independent UK PH cohort, the Scottish Composite Score (SCS) was predictive of survival and able to provide further risk stratification in WHO FC III patients. It may perform better in UK populations than other published equations derived from PH cohorts in France and the United States.

In conclusion, IGR haemodynamic measurements may be useful as alternative outcome measures to 6MWD, and the SCS shows promise as the first UK-based composite risk score in PH. Further studies in larger cohorts are warranted to confirm their clinical utility.

1 INTRODUCTION

This chapter aims to discuss the background and the rationale of the work undertaken for this thesis. It begins with an overview of PH focusing on its clinical classification, pathobiology, pathophysiology, natural history and current outcome measures. Following this is a discussion on the use of cardiopulmonary exercise testing (CPET) and prognostic algorithms in PH. Finally, the hypotheses and aims of this thesis are outlined.

1.1 Overview of pulmonary hypertension

PH is defined as elevated mean pulmonary artery pressure (mPAP) ≥25 mmHg at rest as measured by RHC. This could be caused by a number of conditions directly or indirectly affecting the pulmonary vasculature. Its clinical characteristics and prognosis were first defined in a prospective National Institutes of Health (NIH) study of 198 patients treated across 32 centres in the United States in 1980s [1]. In that era, there was no effective pharmacological therapy and the median survival was only 2.8 years. Two decades on, substantial progress has been made in the understanding of PH pathobiology and pathophysiology. More importantly, over 20 randomised controlled trials of pulmonary vasodilatory therapy have been conducted leading to an expansion of therapeutic options. However PH remains an incurable condition associated with high morbidity and mortality [2].

1.1.1 Clinical classification

The first case of PH was described in 1891 and the name "primary pulmonary hypertension" was first used in 1951 [3]. Since then, the clinical classification of PH has evolved as a result of increased understanding of its natural history. The most recent review of PH management took place at the 4th World Symposium on Pulmonary Hypertension in 2008. The updated classification from this meeting classifies PH into five WHO groups based on the underlying mechanisms of disease [4].

WHO Group 1 **Pulmonary arterial hypertension**

- 1.1 Idiopathic
- 1.2 Heritable
- 1.3 Drugs and toxins induced
- 1.4 Associated with
  - 1.4.1 Connective tissue diseases
  - 1.4.2 Portal hypertension
  - 1.4.3 Congenital heart disease
  - 1.4.4 Human immunodeficiency virus infection
  - 1.4.5 Schistosomiasis
  - 1.4.6 Chronic haemolytic anaemia

- 1’ Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis

WHO Group 2 **Pulmonary hypertension due to left heart disease**

WHO Group 3 **Pulmonary hypertension due to lung diseases and/or hypoxia**

WHO Group 4 **Chronic thromboembolic pulmonary hypertension**

WHO Group 5 **Unclear and/or multifactorial mechanisms**

WHO group 1 pulmonary arterial hypertension (PAH) includes idiopathic PAH which was formerly known as primary pulmonary hypertension and other conditions with similar clinical presentation and pathological changes in the pulmonary microcirculation. This is the group of patients who have been the subjects of most randomised controlled trials of pulmonary vasodilatory therapy, epidemiological studies and basic science research.

PH is also classified into precapillary and postcapillary according to the underlying haemodynamic profile. In precapillary PH, mPAP is ≥25 mmHg, CO is normal or reduced, and pulmonary capillary wedge pressure (PCWP) is ≤15 mmHg. In postcapillary PH, mPAP is ≥25 mmHg, CO is normal or reduced, and PCWP is >15 mmHg. This haemodynamic definition aims to distinguish between conditions that directly affect the pulmonary vasculature resulting in increased PVR, and those that cause raised pulmonary venous pressure leading to a passive

increase in mPAP. Precapillary PH includes WHO groups 1,3,4 and 5. Post-capillary PH includes WHO group 2.

Only patients with WHO group 1 PAH and group 4 chronic thromboembolic pulmonary hypertension (CTEPH) were included in the studies of this thesis and are referred as having precapillary PH thereafter. These are the only groups of patients for whom the use of PAH-specific therapy is recommended by contemporary guidelines [2;5].

1.1.2 Pathobiology

In WHO group 1 PAH, a pulmonary arteriopathy affects distal pulmonary arteries of <500 μm in diameter resulting in an elevation of PVR [6;7]. The pathological changes are characterised by thickening and fibrosis of all three layers of the vessel wall leading to luminal occlusion (known as pulmonary vascular remodelling), plexiform lesions (tumour-like lesions of endothelial cells at pulmonary artery bifurcation) and in-situ thrombus formation. The pulmonary veins are unaffected. The precise trigger for these changes is unknown, but is likely to involve a number of cell types and biochemical pathways [8-10]. Endothelial dysfunction is thought to play an important role by over-expressing vasoconstrictive and proliferative mediators such as thromboxane A_2 and endothelin-1, and under-producing vasodilatory and anti-proliferative mediators such as nitric oxide and prostacyclin, thereby leading to excessive vasoconstriction and proliferative pulmonary vascular remodelling. A prothrombotic state may be responsible for in-situ thrombosis which further reduces lumen calibre. Other cell types such as inflammatory cells, smooth muscle cells, fibroblasts and platelets, and plasma mediators such as vasoactive intestinal peptide have also been implicated.

The precise pathogenesis of CTEPH is unclear. The primary pathological abnormality is the presence of organised thrombi causing thickening of the vessel wall and luminal occlusion in proximal pulmonary arteries. In some patients, there is an additional distal arteriopathy affecting occluded and non-occluded subsegmental pulmonary arteries indistinguishable from that seen in

WHO group 1 PAH [11]. CTEPH may occur as a sequelae of acute pulmonary embolism. Non-resolution of the thrombotic mass leads to mechanical obstruction and pulmonary vascular remodelling through increased shear force, pressure elevation and endothelial dysfunction in both occluded and non-occluded areas. The incidence of CTEPH after the first thromboembolic episode was estimated to be around 4% at 2 years in a prospective long-term follow-up study [12]. However, CTEPH may also develop in the absence of a previous thromboembolic episode. A recent multicentred international CTEPH registry study showed that around 30% of patients did not have a history of acute pulmonary embolism [13]. In such patients, the process of pulmonary vascular remodelling may be initiated by thrombotic or inflammatory lesions in the pulmonary vasculature. Clotting abnormalities involving Factor VIII, platelets and anti-phospholipid antibodies may also play a part [14].

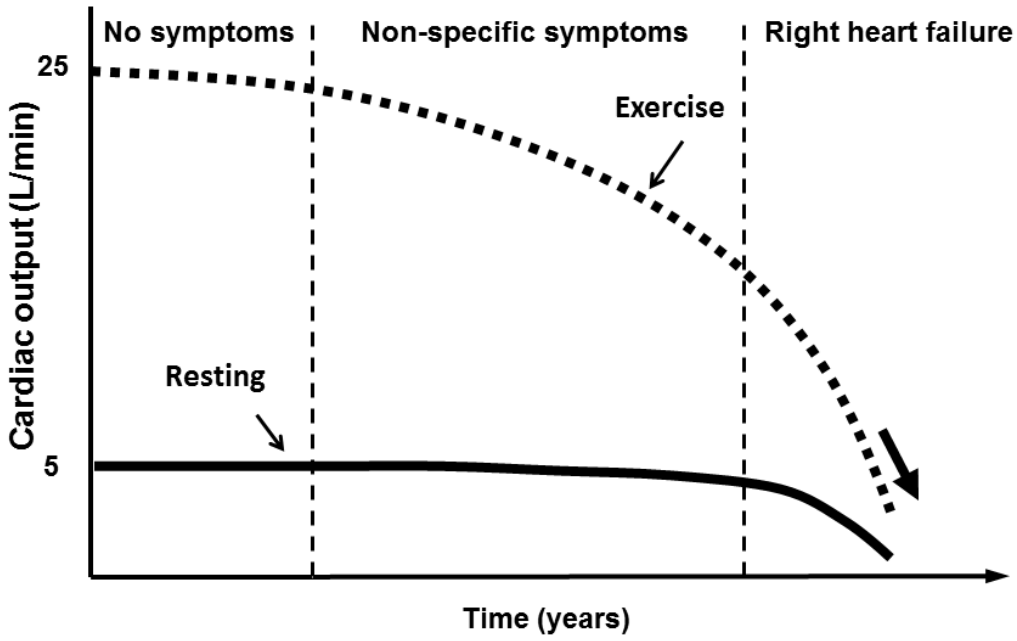
Currently available pulmonary vasodilatory therapy targets three biochemical pathways aimed to ameliorate pulmonary vasoconstriction and pulmonary vascular remodelling. Endothelin receptor antagonists (bosentan, ambrisentan) counteract the vasoconstrictive and proliferative activities of endothelin-1. Phosphodiesterase-5 inhibitors (sildenafil, tadalafil) and prostacyclin analogues (epoprostenol, treprostinil, iloprost) potentiate the vasodilatory and anti-proliferative activities of nitric oxide and prostacyclin. The efficacy of these agents in WHO group 1 PAH has been proven in over 20 randomised controlled trials, and this has been extrapolated to support their off-label use in CTEPH patients. Great efforts are underway to develop new therapeutic agents that would reverse or prevent PH. Those under investigation include tyrosine kinase inhibitors and guanylate cyclase agonists.

1.2 Pathophysiology and natural history

Elevated PVR results in increased impedance to PBF and increased right ventricular afterload. The success of right ventricular adaptation is the main determinant of clinical outcome and survival [15]. In early disease, vasodilatation of non-diseased pulmonary capillary beds compensates for the loss of functional beds elsewhere, thereby maintaining PVR and pulmonary artery pressures.

Patients often have few symptoms as SV and CO at rest and during exercise are preserved. The negative impact on cardiopulmonary function becomes clinically apparent when around 70% of the pulmonary capillary beds are occluded. Patients experience increasing exertional limitation as SV and CO response to exercise become progressively restricted. In advanced disease, CO eventually becomes compromised at rest resulting in overt right heart failure, cardiovascular collapse and death (figure 1.1).

Figure 1.1. Natural history of pulmonary hypertension



Patients are asymptomatic when both resting and exercise cardiac output are preserved. Non-specific symptoms develop on exertion when exercise cardiac output becomes restricted without affecting resting cardiac output. Right heart failure ensues when resting cardac output is also compromised.

1.2.1 Right ventricular structure and function

It was previously thought that the right ventricle (RV) simply existed as a conduit to transfer systemic venous blood to the lungs and the cardiovascular system can function adequately without RV ejection [16]. This was recognised to be untrue as congenital RV hypoplasia would result in circulatory collapse and premature death. Recent advances in imaging techniques such as echocardiography and magnetic resonance imaging (MRI) have allowed sophisticated studies of RV structure and function, and aided the understanding of RV pathophysiology in PH.

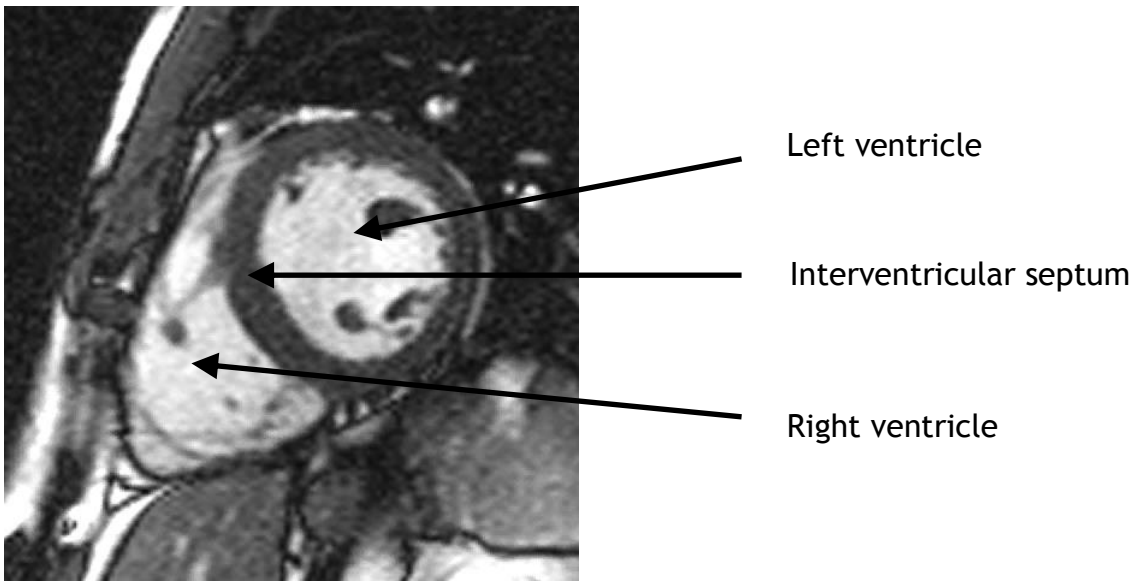
The RV is the most anteriorly placed cardiac chamber behind the sternum. It has a larger volume but smaller muscle mass than the left ventricle (LV) [17]. In contrast to the ellipsoidal shape of the LV, it is triangular when viewed from the side and crescent shaped in cross-section (figure 1.2) [18]. It can be divided into three parts: 1) the inlet portion consisting of the tricuspid valve, chordae tendineae and papillary muscles; 2) trabeculated apical myocardium; and 3) the infundibulum consisting of smooth myocardium and the pulmonary valve. Under normal conditions, LV pressures are higher than RV pressures during both systole and diastole, so the interventricular septum (IVS) bows into the RV under a positive left-to-right trans-septal pressure gradient throughout the cardiac cycle [18].

The ventricles are composed of multiple layers of muscle fibres arranged in an interlacing fashion [19]. The RV free wall is made up of deep and superficial muscle layers. The deep fibres run longitudinally from base to apex and are continuous with those of the IVS. The superficial fibres are arranged circumferentially parallel to the atrio-ventricular groove. They turn obliquely near the apex and continue into the superficial layer of the LV. In comparison, the LV wall is composed of superficial obliquely orientated muscle fibres, sub-endocardial longitudinal fibres and intervening circumferential fibres. Right ventricular contraction starts at the inlet portion, and spreads sequentially to the trabeculated myocardium and infundibulum [20]. Shortening of longitudinal fibres causes the tricuspid annulus to move towards the apex, and that of

circumferential fibres produces inward movement of the RV free wall. There is a greater degree of longitudinal than circumferential shortening. In contrast, LV contraction is concentric involving twisting and rotation in addition to shortening, due to the action of oblique fibres. The continuous nature of ventricular wall musculature allows mechanical interaction between ventricles throughout the cardiac cycle, known as "ventricular interdependence" [20].

The RV performs differently from the LV due to differences in muscle mass, chamber geometry and orientation of myocardial fibres [21-23]. The RV has a smaller ejection fraction and less contractile reserve than the LV, and is therefore more sensitive to changes in afterload [24;25]. Comparison between LV and RV is summarised in table 1.1.

Figure 1.2. Two-chamber view of a normal heart by magnetic resonance imaging



The left ventricle is spherical and the right ventricle triangular when viewed from the side.

Table 1.1. Comparison of left and right ventricular structure and function

	Right ventricle (RV)	Left ventricle (LV)
Structure	inflow region, tracbeculated apical myocardium and infundibulum	inflow region, myocardium and outflow tract
Shape	triangular from the side, crescent shaped in cross-section	ellipsoidal
Mass	One sixth of LV	-
Wall thickness, mm	2 to 5	7 to 11
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
Ventricular pressure, mmHg	average 25/4	average 130/8
Compliance at end-diastole	higher	lower
Filling profiles	starts earlier and finishes later lower filling velocities	starts later and finishes earlier higher filling velocities
End-diastolic volume, ml/m^2	75±13	66±12
Ejection fraction, %	61±7	67±5
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 [26].

1.2.2 Right ventricular response to increased afterload

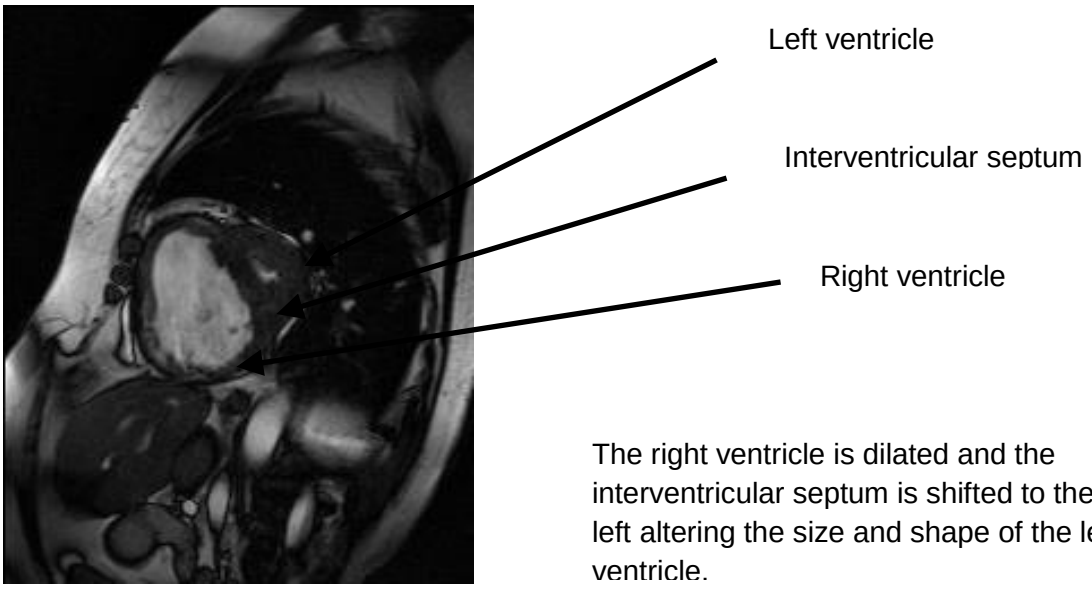
Right ventricular adaption to increased afterload determines the clinical course and outcome in PH. This adaptive process, consisting of dilatation, hypertrophy and a compensatory increase in contractile performance, is influenced by several factors including the time course of disease, the age of onset (congenital versus adult-onset heart disease) and the pattern of RV remodelling. Right ventricular failure occurs early in situations such as acute pulmonary embolism where RV adaptation is outpaced by an acute and rapid increase in afterload from a significant embolic burden [27]. In congenital heart disease (CHD) associated with left-to-right shunting, longstanding exposure to systemic blood pressure causes the RV to undergo hypertrophic growth in parallel with the LV from birth. When PH and consequent reversal of shunt develop, the appropriately hypertrophied RV is able to maintain its systolic function against an increased afterload for decades before the onset of RV failure. The preservation of RV function accounts for the superior survival of these patients over those with other forms of PH [28-30]. The pattern of RV remodelling is also influenced by altered gene expression, neurohormonal and cytokine activation as some patients develop RV failure earlier than others with the same degree of PH [31].

1.2.3 Pathophysiology of right ventricular failure

In the early stages of pulmonary vascular disease, RV responds to an increased afterload by undergoing concentric hypertrophy [32]. The muscle mass increase results from increased protein synthesis and cardiomyocyte size through the addition of sarcomeres in response to increased systolic and diastolic stretch caused by rising systolic and diastolic ventricular pressures [33]. This adaptive hypertrophy serves to enhance systolic contraction and maintain CO but can only be sustained for a finite period of time. It eventually gives way to ventricular dilatation as disease progresses. The mechanisms initiating this switch are unclear but there is increasing evidence to support the importance of oxygen demand/supply imbalance [34], resulting in complex changes in the cardiomyocytes and their extracellular matrix [33]. Right ventricular dilatation is

associated with disruption of the normal pressure-volume relationship and a progressive decline in contractility. It also results in greater right ventricular wall tension which further compromises contractility by increasing myocardial oxygen demand and reducing ventricular perfusion. As RV systolic function is reduced, contraction time becomes prolonged resulting in interventricular mechanical asynchrony (RV is still contracting as LV enters diastole) [35-37]. This causes leftward bowing the IVS into the LV cavity during early diastole under a positive right to left trans-septal pressure gradient (figure 1.3) and reduction in left ventricular end-diastolic volume. Combined with reduced RV SV, it leads to impaired left ventricular diastolic filling and consequently a marked decline in CO seen in PAH. RV dilatation also causes functional tricuspid regurgitation through annular dilatation and chordal traction, which further worsens right ventricular volume overload [38]. Additional factors that have been implicated in the development of right heart failure include sympathetic activation [39;40], oxidative stress, immune activation and cardiomyocyte apoptosis [33]. Therefore the development of RV dilatation signals the beginning of a vicious cycle of events culminating in right heart failure.

Figure 1.3. Leftward septal bowing in right ventricular volume overload



The right ventricle is dilated and the interventricular septum is shifted to the left altering the size and shape of the left ventricle.

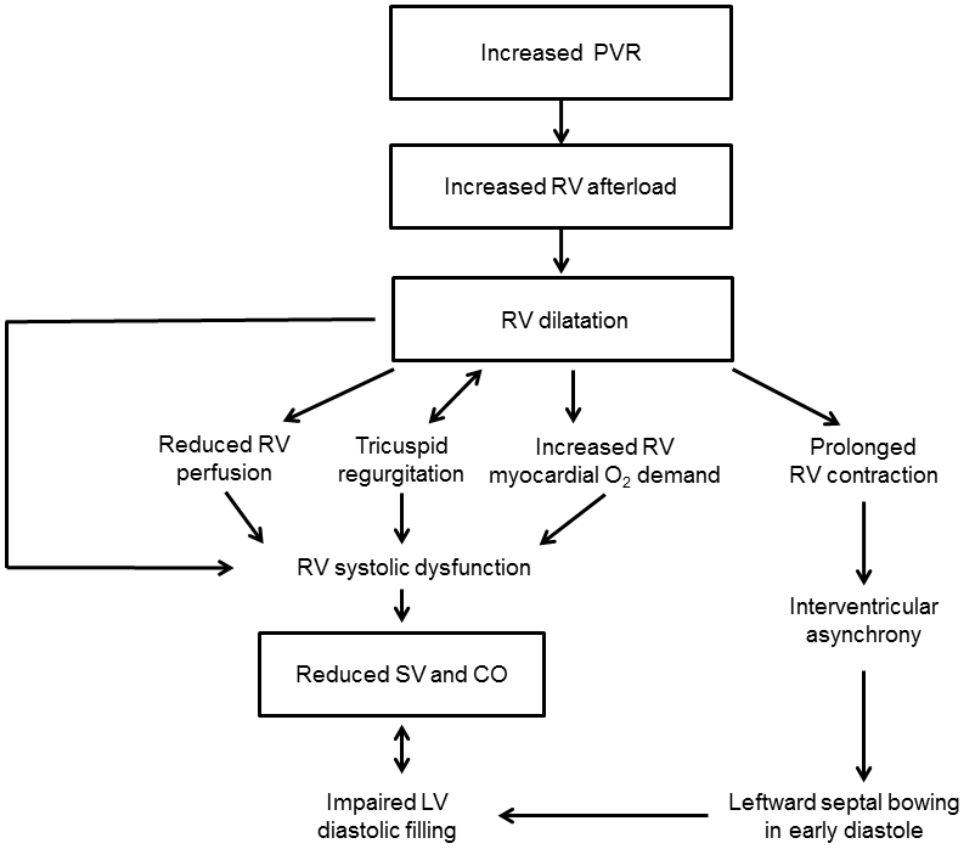
1.2.4 Compensatory changes in systemic oxygen transport

The direct consequence of a restricted exercise SV and CO response is impaired systemic oxygen delivery to exercising muscles. This is partially compensated by increased oxygen extraction from the peripheral blood. As a result, the arterio-venous oxygen content difference ($C_{a-v}O_2$) is widened and mixed venous oxygen saturation (S_vO_2) falls [41]. This relationship between CO, oxygen uptake (VO_2) and $C_{a-v}O_2$ is described by the Fick Principle.

$$VO_2 = CO \times C_{a-v}O_2$$

Peak VO_2 is reduced despite a compensatory increase in $C_{a-v}O_2$ and is manifest as impaired exercise capacity. A low S_VO_2 reflects a low CO state and is associated with poor prognosis [42-44].

Figure 1.4. Pathophysiology of right ventricular failure



CO: cardiac output; LV: left ventricle; PVR: pulmonary vascular resistance; RV: right ventricle; SV: stroke volume; O_2: oxygen.

1.3 Outcome measures in pulmonary hypertension

The availability of effective pulmonary vasodilatory therapy has expanded considerably since the efficacy of the first agent, intravenous epoprostenol, was proven in a randomised controlled trial two decades ago [45]. After a 12-week treatment period, actively treated patients were shown to have improved 6MWD, pulmonary haemodynamics, quality of life (QOL) and survival compared with those on conventional therapy only. With identification of new molecular targets, eight oral agents have since been developed and their efficacy proven in randomised controlled trials using change in 6MWD as the primary end-point [2]. Other co-primary or secondary end-points include WHO FC, pulmonary haemodynamics, brain natriuretic peptide (BNP) and NT-proBNP, health-related QOL and time to clinical worsening (TTCW).

1.3.1 Six-minute walk distance

Exercise assessment is an integral part of disease management in PH. Lowering of PVR by pulmonary vasodilatory therapy would off-load the RV and improve CO response to exercise. Exercise capacity is therefore directly linked to right heart function. Depending on the measurements of interest, exercise assessment could be undertaken in a variety of forms: field tests versus laboratory-based tests, incremental exercise versus endurance exercise, treadmill testing versus cycle ergometry. The principal form of exercise assessment adopted in PH is the 6MWT which measures the distance walked in 6 min [2]. The distance covered is proportional to the maximal average walking speed achieved over this period of time, and therefore represents the maximal sustainable aerobic exercise capacity. It correlates with peak VO_2 and oxygen pulse (VO_2/HR) measured at CPET [46], and metabolic equivalent measured at treadmill testing [47]. Unlike these laboratory-based tests, 6MWT can be performed in a hospital corridor without the need for technical expertise or sophisticated equipment. It has been shown to be reproducible with <10% change on initial repeated testing [48]. International guidelines on the standardisation of 6MWT exist to ensure results are interpretable across centres [49].

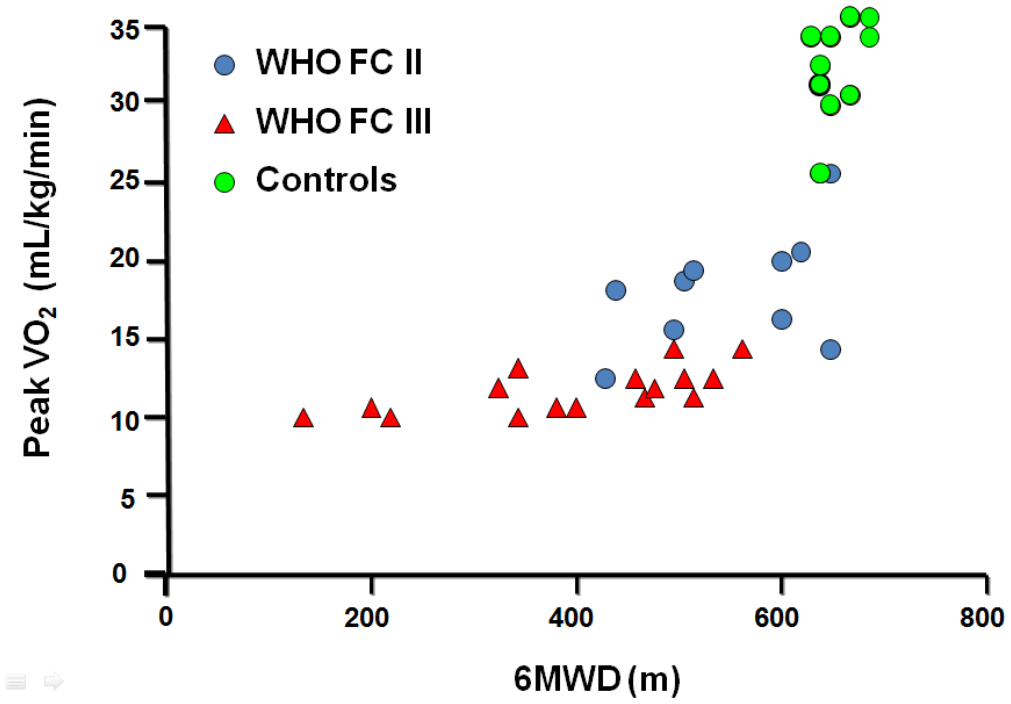
6MWD correlates with other markers of disease severity such as pulmonary haemodynamics and WHO FC [46]. Since its ability to detect a treatment effect and survival benefit was first demonstrated in the landmark trial of intravenous epoprostenol [45], 6MWD has been used as a primary end-point in most randomised controlled trials of currently available oral pulmonary vasodilatory agents [50-56]. Its prognostic value was subsequently confirmed in a series of observational studies on the long-term effects of PAH-specific therapy [57-60]. Currently, 6MWD is the only exercise end-point accepted by the Food and Drug Administration in the Unites States and European Agency for the Evaluation of Medicinal Products to study the effects of pulmonary vasodilatory therapy. However, it is increasingly recognised that there are limitations to 6MWD that compromise its reliability as the primary end-point, and there is a need to develop alternative outcome measures that are more robust and disease-specific [61;62] as the treatment strategy for PH continues to evolve.

As with other physiological measurements such as lung function and cardiopulmonary exercise capacity, 6MWD varies greatly in healthy individuals due to age, gender, height and weight. Meaningful interpretation of abnormal values can only be made in the context of known normality. Although several reference equations have been developed to correct for these physiological factors and predict normal values [63-66], there is no standardisation on which equation to use and how 6MWD results should be presented. Prognostic 6MWD thresholds identified in different studies are all based on absolute values [46;60]. This makes interpretation difficult in patients at the extremes of age and body habitus. Percent predicted 6MWD (% predicted 6MWD) could give a more objective measure of deviation from normality, but there are so far no published data on its use in the clinical evaluation of PH patients.

Performance at the 6MWT can be influenced by a number of factors unrelated to PH, such as co-existing cardiopulmonary, neurological and musculoskeletal problems, subject volition and walking efficiency [49]. These factors may be more important determinants of 6MWD than the cardiopulmonary effect of PH such that any treatment-induced change in exercise capacity may be masked. The validity of 6MWD as an end-point in patients with milder disease (WHO FC I and

II) has also been called into question. In the EARLY (Endothelin Antagonist tRial in mildLY symptomatic pulmonary arterial hypertension) trial, 6 months of bosentan treatment in WHO FC II patients had no significant effect on 6MWD despite reducing PVR (co-primary end-point) and TTCW (secondary end-point) [67]. Another study showed that PAH-specific therapy induced an improvement in haemodynamic measurements and WHO FC, but had no significant effects on 6MWD in patients walking >450 m at diagnosis [68]. These findings suggest that 6MWD loses sensitivity as an outcome measure in fitter patients or those with early disease presumably due to a ceiling effect. During the walk test, VO_2 increases initially and reaches a plateau as the subject reaches the maximal average walking speed [69]. VO_2 cannot increase further beyond this point as the subject is not permitted to run. Hence 6MWD becomes disconnected with maximal exercise capacity as measured by peak VO_2. This ceiling effect is illustrated in a study of healthy subjects and heart failure patients where a curvilinear relationship between peak VO_2 and 6MWD was demonstrated with overlapping of 6MWD between patients in WHO FC II and healthy controls (figure 1.6) [70]. Any improvement in exercise capacity would be less evident when measured by a change in 6MWD if initial 6MWD approaches the normal range.

Figure 1.5. Ceiling effect of six-minute walk distance



Modified from Lipkin DP et al. B Med J 1986;292:653-655 [70]; 6MWD: six-minute walk distance; VO_2: oxygen uptake; WHO FC: World Health Organisation functional class.

1.3.2 Other outcome measures

Pulmonary haemodynamics

Patient outcome is closely linked to the severity of pulmonary haemodynamic impairment. Right atrial pressure (RAP) [1;43;44;59;60;71;72], mPAP [1;60], CO [73], PVR [71] and S_vO_2 [42;57] have all been shown to predict survival with RAP and CO providing the strongest prognostic signals. These haemodynamic indices also correlate with WHO FC and exercise capacity, but the relationships are not tight. Miyamoto et al found that there was no significant correlation between mPAP and 6MWD [46], which could be explained by the fact that mPAP may fall as RV fails in advanced disease. Kawut et al found that despite having similar pulmonary haemodynamics, patients with connective tissue disease associated pulmonary arterial hypertension (CTDPAH) had worse prognosis than those with idiopathic pulmonary arterial hypertension (IPAH) [74]. The conventional method of calculating PVR based on resting measurements is also problematic. PVR is derived by the quotient of driving pressure (difference between mPAP and PCWP) and flow (CO). If only single point measurements at rest are used, the gradient of the pressure-flow relationship may be under- or over-estimated due to the assumption that it is linear and crosses zero [75;76]. This relationship could be more reliably determined by taking multi-point measurements at rest and then on exercise, and defining incremental PVR as the slope of the pressure-flow plot [77]. Provencher et al demonstrated that exercise haemodynamics were more closely linked with therapy-induced improvement in exercise capacity than resting haemodynamics [78]. The findings from this study further highlight the importance of exercise assessment in PH.

World Health Organisation functional class

WHO FC has been used to define functional status in PH patients and is a strong prognostic marker. Historical data on untreated patients with idiopathic or heritable PAH showed that the median survival was 6 months for WHO FC IV, 2.5 years for WHO FC III and 6 years for WHO FC I and II [1]. A more recent study on the long-term outcome of IPAH patients receiving intravenous epoprostenol showed that patients in WHO FC IV at baseline had worse prognosis than those in

FC III, and patients whose FC improved to I and II after treatment had better survival than those remaining in FC III and IV [60]. In current practice, WHO FC is a vital part of clinical evaluation to assess stability and prognosis.

N-terminal pro-brain natriuretic peptide

NT-proBNP has emerged as the biomarker of RV function/dysfunction in recent years. It is released when BNP precursor is cleaved to form BNP in response to increased ventricular wall tension. Measurement of NT-proBNP is preferred to BNP as NT-proBNP is more stable in the peripheral blood and hence less susceptible to the influence of posture or activities. Baseline and/or follow-up NT-proBNP/BNP levels have been shown to correlate with survival and other markers of disease severity such as WHO FC, pulmonary haemodynamics, 6MWD [79-82]. Serial BNP/NT-proBNP measurements may be used to track RV function in deteriorating or improving patients during follow-up [83]. In one study where the efficacy of pulmonary thromboendarterectomy in CTEPH patients was assessed by BNP, post-operative improvement in pulmonary haemodynamics was paralleled by a fall in BNP levels, and the levels remained elevated in patients with residual PH [84]. NT-proBNP has been included as a secondary end-point in recent clinical trials of PAH-specific therapy, and significant decreases in its level were observed in the treatment group versus placebo group [54;67]. Further large scale studies are awaited to confirm its usefulness as a biomarker of RV function.

Quality of life measures

The impact of PH and its treatments on patient QOL has not been systematically studied in clinical practice. Generic QOL measures have been included as secondary end-points in some clinical trials of PAH-specific therapy such as the 36-item Short-Form Health Survey, European Quality of Life Scale, and Minnesota Living with Heart Failure Questionnaire. The content of these measures is not disease-specific and hence may not adequately describe the specific symptoms experienced by PH patients. A new PH-specific QOL assessment tool, the CAMPHOR score, has been developed by a group in Cambridge based on interviews of 35 PH patients and analysis of their responses

to PH-specific questions [85]. It has been shown to have good internal consistency and test re-test reliability. However, before CAMPHOR score could be widely used in multi-national clinical trials, adaptations for different languages and countries need to be developed and validated. Its routine use in the clinical evaluation of PH patients is recommended by current UK and Ireland PH guidelines [5].

Time to clinical worsening and survival

Contrary to the survival benefit shown in the epoprostenol trial [45], a meta-analysis of 16 randomised clinical trials of pulmonary vasodilatory agents conducted between 1985 and 2005 including 1962 patients found that treatments produced a modest improvement in exercise capacity (a mean change in 6MWD of 42.8 m), but this change was not predictive of survival [86]. On the other hand, a more recent meta-analysis of 21 randomised controlled trials including 3140 patients conducted between 1990 and 2008 demonstrated active treatments conferred a 43% relative reduction in mortality [87]. Although several registry studies have demonstrated improved survival of patients treated with modern PAH-specific therapy compared with historical cohorts [71-73;88], the survival benefit of PAH-specific therapy (other than intravenous epoprostenol) has not been proven directly in randomised controlled trials. In order to address this issue, future clinical trials should ideally use mortality as the primary end-point, but this approach would require large sample sizes and relatively long study periods [89]. A composite end-point, TTCW, has been developed as a surrogate of mortality to overcome this. It is defined as the time to the first adverse event, which may include 1) all-cause mortality; 2) hospitalisation due to PH; 3) the need for additional PAH-specific therapy; 4) the need for lung transplantation or atrial septostomy and 5) clinical progression of PH. It has been successfully used to demonstrate the efficacy of treatments in more recent clinical trials [54;90;91].

1.4 Cardiopulmonary exercise testing in pulmonary hypertension

Patients affected by PH typically present with exercise intolerance due to breathlessness, fatigue, chest pain and/or pre-syncope. CPET allows a detailed and non-invasive examination of the integrated response to exercise from the cardiovascular, respiratory and musculoskeletal systems. It provides insights into the mechanisms of exercise limitation, and helps to differentiate between pulmonary vascular and other causes in patients with comorbid conditions. Its safety and reproducibility have been demonstrated in patients with PH [92]. Unlike the 6MWT, it is not subject to a ceiling effect, and may be more discriminatory in assessing younger patients or those with early disease in whom 6MWD may be preserved [70;93]. More importantly, several CPET variables have been shown to predict survival in PH [94] and have the potential to be used as markers of therapeutic response. Current guidelines recommend that CPET should be part of routine diagnostic and prognostic assessment in PH [2].

1.4.1 Cardiopulmonary exercise profile in pulmonary hypertension

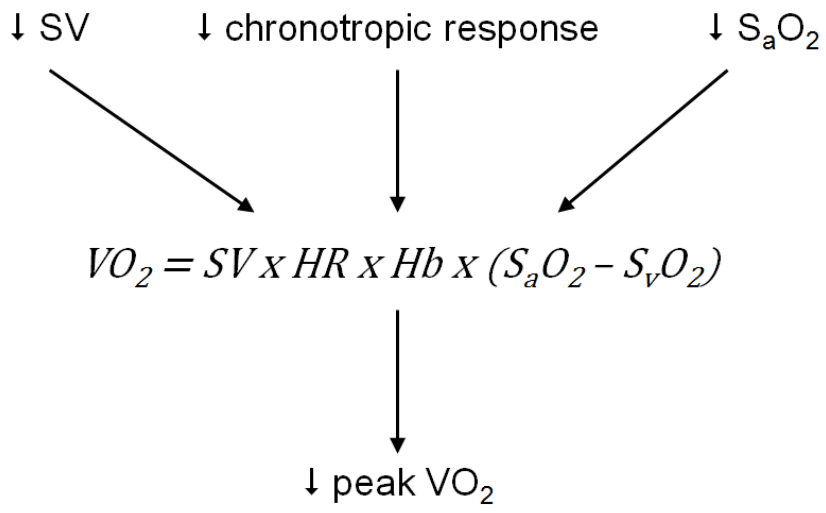
PH impacts negatively on exercise capacity through its effects on oxygen transport and pulmonary gas exchange [95], which could be assessed by measuring specific variables at CPET (table 1.2).

Oxygen transport (figure 1.6)

In PH, oxygen delivery to peripheral muscles is reduced due to impaired SV augmentation on exercise. Muscle work is more dependent on anaerobic metabolism, giving rise to an early anaerobic threshold (AT) (reached at less than 50% of peak VO_2) and reduced work efficiency measured by the gradient of VO_2/work rate relationship (VO_2/WR slope). Peak VO_2 and peak VO_2/HR, respective markers of maximal CO and SV, are reduced. There is a greater dependence on heart rate (HR) increase to augment CO during exercise, giving rise to an increased HR/VO_2 slope. On the other hand, chronotropic response to exercise is impaired due to autonomic dysfunction [96] and peak HR is often not

reached. Oxygen transport is further compromised if profound hypoxaemia develops during exercise.

Figure 1.6. Oxygen transport in pulmonary hypertension



Hb: haemoglobin concentration; HR: heart rate; S_aO_2: arterial oxygen saturation; S_vO_2: mixed venous oxygen saturation; SV: stroke volume; VO_2: oxygen uptake.

Pulmonary gas exchange (figure 1.7)

The gas exchange abnormalities in PH are characterised by V/Q mismatch with increased perfusion to low V/Q units, increased dead space and a shift of the mean V/Q ratio to higher than normal (>1), leading to increased $P_{A-a}O_2$ (and consequently hypoxaemia) and ventilatory inefficiency [97]. Hypoxaemia is compounded by a low S_vO_2 due to increased peripheral oxygen extraction caused by a low CO, which has the greatest effects in the presence of low V/Q units and shunts. Increase in dead space to tidal volume ratio (V_D/V_T) leads to wasted ventilation, thereby increasing the ventilatory requirement to eliminate a given amount of carbon dioxide produced from metabolism and hence reducing ventilatory efficiency. This is recognised by an increased minute ventilation to carbon dioxide output ratio (V_E/VCO_2) at AT (also known as the ventilatory equivalent for carbon dioxide), an increased gradient of the V_E/VCO_2 relationship (V_E/VCO_2 slope) and reduced end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) at AT.

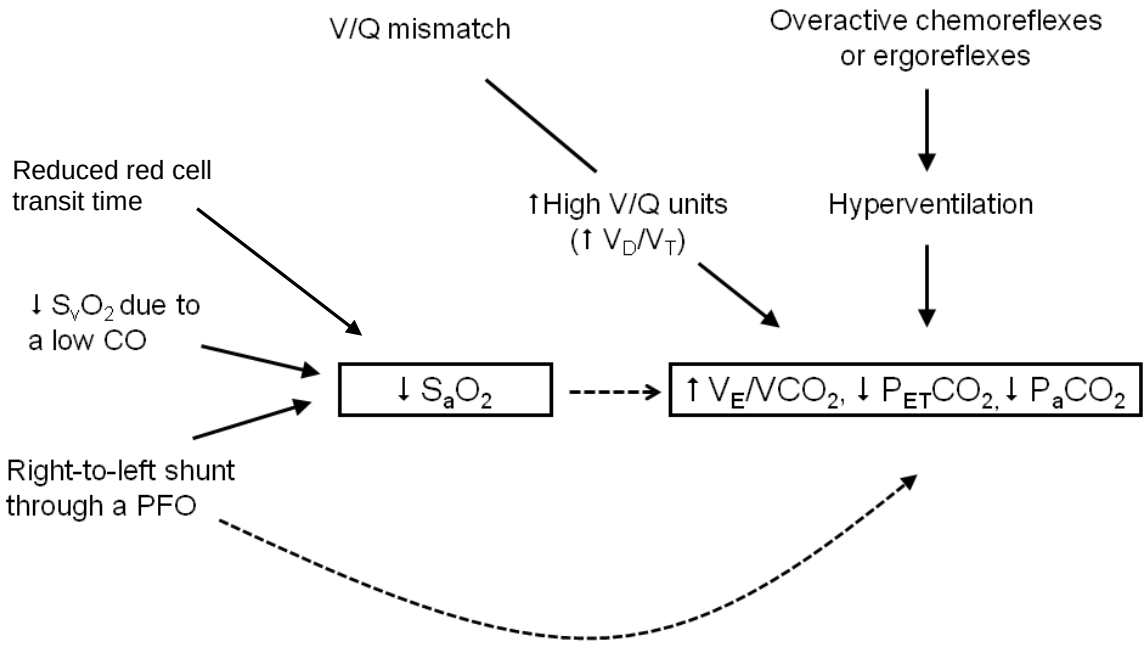
Patients with PH hyperventilate at rest and during exercise out of proportion to VCO_2 resulting in hypocapnia which has been associated with a poor prognosis [98]. The disproportionate increase in ventilation is thought to be linked to sympathetic nervous system activation mediated by overactive ergoreflexes and chemoreflexes in exercising muscles, but the precise mechanisms remain incompletely understood [99]. The relationship between V_E, VCO_2, P_aCO_2 and V_D/V_T is defined by the modified alveolar ventilation equation.

$$V_E/VCO_2 = \frac{863}{P_aCO_2 \times (1 - V_D/V_T)}$$

During exercise, V/Q matching does not deteriorate significantly but ventilatory efficiency worsens with a further increase in the mean V/Q ratio. Hypoxaemia occurs primarily as a result of reduced S_vO_2 caused by a low CO. Ventilatory response is further heightened if peripheral chemoreceptors are stimulated by profound hypoxaemia resulting from right-to-left shunting through a patent foramen ovale (PFO). This can be recognised at CPET with an overall accuracy of

90-96% by an abrupt and sustained decrease in $P_{ET}CO_2$, a concurrent abrupt and sustained increase in end-tidal oxygen partial pressure ($P_{ET}O_2$), an abrupt and sustained increase in respiratory exchange ratio (RER) and arterial oxygen desaturation [100].

Figure 1.7. Pulmonary gas exchange in pulmonary hypertension



PFO: patent foramen ovale; P_aCO_2: arterial carbon dioxide partial pressure; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; S_aO_2: arterial oxygen saturation; S_vO_2: mixed venous oxygen saturation; V/Q: ventilation/perfusion; V_D/V_T: dead space to tidal volume ratio: V_E/VCO_2: ventilation equivalent for carbon dioxide.

Table 1.2. Cardiopulmonary exercise characteristics in pulmonary hypertension

Oxygen transport	Reduced peak VO_2 Reduced VO_2 at AT Reduced VO_2/HR Increased HR/VO_2 slope Reduced peak HR Reduced VO_2/WR slope towards peak exercise
Pulmonary gas exchange	Increased V_E/VCO_2 at AT Increased V_E/VCO_2 slope Reduced $P_{ET}CO_2$ at AT Increased V_D/V_T Increased $P_{A-a}O_2$ gradient

AT: anaerobic threshold; HR: heart rate; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; $P_{A-a}O_2$ gradient: alveolar-arterial oxygen partial pressure gradient; V_E: minute ventilation; VCO_2: carbon dioixde output; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_D/V_T: dead space to tidal volume ratio; V_E/VCO_2: ventilatory equivalent for carbon dioxide output; WR: work rate.

1.4.2 Clinical applications

CPET is useful in providing an objective assessment of maximal exercise capacity and identifying patients who demonstrate a pulmonary vascular limit to exercise for further diagnostic evaluation. Once the diagnosis of PH is confirmed, the main applications of CPET lie in the areas of therapeutic and prognostic assessments.

Therapeutic assessment

Published data on the use of CPET variables to assess response from therapeutic interventions are primarily from studies on patients with COPD. Most of these studies looked at changes in peak VO_2, VO_2 at AT, peak HR and V_E during incremental CPET. The results were equivocal with some studies showing

modest improvement in peak VO_2 [101-103], while others failing to do so [104;105]. Changes in peak HR and V_E with therapy were not observed [103;105;106]. In recent years, the focus of exercise assessment has shifted from incremental symptom-limited protocols to constant-load endurance protocols as a means to detect therapy-induced changes in exercise capacity. In constant-load endurance protocols, subjects perform cycle ergometry at a constant submaximal work rate until exhaustion. High-intensity (i.e. 75-80% of peak VO_2) or moderate-intensity exercise (i.e. below AT) has been used. The measurements of interest are endurance time, defined as the time to symptom limitation, and metabolic variables (such as inspiratory capacity, VO_2, V_E, V_E/VCO_2, and HR) at a standardised time (for instance, after 5 min of exercise). This "isotime" analysis allows assessment of treatment-induced changes in metabolic variables at points of identical physiological stress, and identification of the physiological mechanisms responsible for improved exercise capacity. A positive effect of intervention is indicated by an increase in endurance time or improvements in isotime metabolic variables at identical submaximal work rates. High-intensity constant-load endurance protocols have been used to demonstrate the benefit of interventions such as bronchodilator therapy [107], oxygen [108] and heliox administration during exercise [109], bronchoscopic lung volume reduction surgery [110] and pulmonary rehabilitation in COPD patients [111]. Improvements in endurance time in these studies were accompanied by reductions in dynamic hyperinflation and dyspnoea at isotime. One study compared the effect of bronchodilator therapy on changes in exercise performance measured by three different modes of exercise (6MWT, incremental cycle ergometry and high-intensity endurance cycle ergometry) and showed that there was no significant improvement in peak VO_2, a small increase in 6MWD (1%), and a significant increase in endurance time (19%) [112]. Metabolic measurement during endurance exercise is increasingly used for therapeutic assessments in COPD patients [113].

Incremental CPET variables have been used to assess the effect of pulmonary vasodilatory therapy in PH in eight studies published between 1999 and 2007. A majority of these were uncontrolled single-centre studies including small numbers of patients (<30) except for two multicentre randomised controlled

trials which included >100 patients. In the small studies, intravenous prostacyclin [114], inhaled iloprost [115] and oral sildenafil [116] were shown to alter CPET response. The variables that improved significantly with therapy included peak VO_2, $P_{ET}CO_2$ at AT, V_E/VCO_2 at rest and at AT, and the V_E/VCO_2 slope. On the other hand, the larger scale randomised controlled trials on sitaxentan [117] and beraprost [118] failed to demonstrate an improvement in CPET response despite a significant increase in 6MWD with therapy. On further data analyses, the authors concluded that a lack of standardisation of CPET procedures and variations in CPET experience among study centres may account for the negative results as data from centres with less CPET experience were found to be less reliable [119]. Therefore, it is essential to ensure uniformity of exercise protocols and technical standards in future clinical trials intended to use CPET variables as primary end-points. Although the mechanisms of exercise limitation in patients with PH are distinctly different from those experienced by COPD patients (cardiac versus pulmonary), constant-load endurance exercise may have a role in therapeutic assessments in PH. However, there are so far little published data on the use of endurance time or isotime metabolic variables in detecting treatment response in PH. These measurements may be potentially more sensitive outcome measures than incremental CPET variables or 6MWD.

Prognostic assessment

Current European PH guidelines recommend that CPET be part of baseline evaluation and should be repeated during follow-up to reassess disease severity and prognosis [2]. However, there have been relatively few studies evaluating the prognostic value of CPET variables in PH. Wensel et al first evaluated the ability of CPET responses to predict survival in a group of patients with primary pulmonary hypertension (now classified as idiopathic, heritable and anorexigen-associated PAH) [94]. Peak VO_2 and peak systolic blood pressure (SBP) were shown to be independent mortality predictors at multivariate analysis. Patients with both risk factors (peak VO_2 ≤10.4 ml/kg/min and peak SBP ≤120mmHg) had the worst survival (23% at 1 year). Those with one of these risk factors had intermediate survival (79% at 1 year) and those with no risk factors had the best survival (97% at 1 year). The prognostic significance of CPET responses was

confirmed in another cohort of PH patients with mixed aetiologies [120]. A recent study by Deboeck et al was the first to address the questions if exercise capacity predicts TTCW and if CPET variables are equally predictive in patients with IPAH and those with associated PAH [121]. It showed that in IPAH patients, 6MWD and V_E/VCO_2 at AT predicted survival, and peak VO_2 predicted TTCW independently. The optimal cut-off values to predict survival at 4 years were 54 for V_E/VCO_2 at AT and 307 m for 6MWD, and that to predict TTCW was 11.6 ml/kg/min for peak VO_2. On the other hand, none of the CPET variables or 6MWD predicted survival or TTCW in patients with associated PAH. This may be related to the heterogeneous nature of conditions in the associated PAH group including both patients with relatively good prognosis (CHD) and those with worse prognosis (systemic sclerosis, SSc). The findings in this study have raised uncertainty about the reliability of CPET to assess disease status in patients with co-existing medical conditions that are likely to compound exercise limitation.

1.5 Non-invasive cardiac output measurement during exercise

1.5.1 Importance of cardiac output measurement

The progressive increase in VO_2 during incremental exercise is brought about by a concomitant increase in CO (determined by cardiac function) and $C_{a-v}O_2$ (determined by the ability of exercising muscles to extract oxygen assuming that arterial oxygen content is normal). In healthy individuals, there is a predictable linear relationship between $C_{a-v}O_2$ and relative exercise intensity expressed as % predicted peak VO_2, described by the equation $C_{a-v}O_2$ = 5.72+(0.1x % predicted peak VO_2) [122]. Hence CO could be reliably estimated from VO_2 by applying the direct Fick principle ($CO = VO_2/C_{a-v}O_2$). However, this relationship cannot be applied to patients with cardiopulmonary diseases, and thus CO needs to be measured directly. The value of CO measurement during exercise was first recognised in patients with chronic heart failure (CHF). Several studies showed that CO derived parameters at peak exercise were more closely related to prognosis than peak VO_2 [123-126]. They may also be more sensitive than peak VO_2 in

assessing response from therapeutic interventions, although published data in this area are limited. Furthermore, direct CO measurement may allow clinicians to distinguish patients with poor exercise capacity due to pump failure from those with muscle de-conditioning, hence selecting those who would benefit most from cardiac transplantation.

In PH, 6MWD has been established as an indirect marker of exercise CO due to its simplicity and reproducibility. However, several drawbacks exist that compromise its reliability as an outcome measure in PH, namely loose correlations with other markers of disease severity and loss of sensitivity in detecting clinical change in fitter patients (ceiling effect). Moreover changes in 6MWD are not disease-specific and may not signify an improvement in pulmonary haemodynamics (discussed in Chapter 1.3.1). Therefore it would be of clinical value to measure exercise CO directly as a specific marker of right heart function. The "gold standard" of CO measurement is the direct Fick method whereby CO is calculated as a quotient of VO_2 and $C_{a\text{-}v}O_2$ [127]. VO_2 needs to be measured directly at bedside and determination of $C_{a\text{-}v}O_2$ requires sampling of mixed venous blood from the pulmonary artery at RHC. This has been superseded by the more convenient thermodilution technique (TD) [128], but the invasive nature of RHC remains an obstacle to the use of repeated CO measurements for follow-up assessment in routine clinical practice. Several non-invasive techniques have been developed to measure CO obviating the need for RHC. These include impedance cardiography [129] and pulse contour analysis [130], but they have not been proven useful during exercise in PH.

1.5.2 Inert gas rebreathing method

The IGR method measures PBF during rebreathing of an oxygen enriched mixture of blood-soluble and blood–insoluble gases [131]. As inert gases are non-physiological, their serum concentrations in systemic venous blood can be assumed to be zero. As the subject re-breathes through a respiratory apparatus from a bag prefilled with the gas mixture for about 30 s, the blood-soluble gas dissolves rapidly in the pulmonary capillary blood, and its rate of disappearance from the alveoli is proportion to the effective PBF. The blood-insoluble gas is

not taken up in the pulmonary capillary blood, and remains in the alveoli to correct for changes in total alveolar volume during the rebreathing manoeuvre. Cardiac output is equivalent to PBF in the absence of significant intrapulmonary or intracardiac shunts.

Acetylene rebreathing method using a mass spectrometer has been shown to provide an accurate estimate of CO compared with TD and the Fick method in PH patients [132]. Reliability and reproducibility of CO measured by a more recently developed metabolic system (Innocor, Innovision, Odense, Denmark) using rebreathing of nitrous oxide (N_2O) and sulphur hexafluoride (SF_6) has been demonstrated in PH patients and patients with interstitial lung disease [133;134]. Compared with a mass spectrometer, this new system is more portable and easier to maintain. It also has the added advantage of providing simultaneous metabolic measurements at rest and during exercise. Its ease of use has been demonstrated in CHF patients [135;136], but this remains to be explored in PH patients.

1.6 Use of prognostic algorithms in pulmonary hypertension

Accurate prognostic assessment is integral to optimal management in PH as it guides treatment decisions and patient counselling. Observational studies on the long-term outcome of patients treated with PAH-specific therapy [58-60;137;138] and registry studies from different treatment eras [1;71-73] have demonstrated the prognostic significance of a multitude of clinical, functional and laboratory variables. A recent systemic review found that most factors that prognosticate mortality in IPAH were assessed in very few studies, and there is discrepancy between studies on the prognostic value of many factors [139]. Only 10 out of 107 factors identified had a reproducible association with mortality demonstrated in more than three studies. These include WHO FC, HR, 6MWD, pericardial effusion, mPAP, RAP, cardiac index (CI), stroke volume index, PVR and S_vO_2.

The current European PH guidelines laid out clear recommendations on the use of established prognostic parameters to assess disease severity and predict patient outcome [2]. These parameters reflect the impact of disease on symptoms (clinical evidence of RV failure, syncope, WHO FC), exercise capacity (6MWD and CPET indices) and RV function (BNP/NT-proBNP, echocardiography and pulmonary haemodynamics), and can be used to stratify patients into good and poor prognostic groups (table 1.3). For patients with intermediate abnormalities, other factors such as age, PH aetiology and comorbidities should also be taken into consideration.

Table 1.3. Established prognostic parameters to assess disease severity and prognosis in pulmonary hypertension [2]

Determinants of prognosis	Better prognosis	Worse prognosis
Presence of RV failure	no	yes
Progression of symptoms	slow	rapid
Syncope	no	yes
WHO FC	I, II	IV
6MWD	>500 m*	<300 m*
CPET	Peak VO_2 >15 ml/min/kg	Peak VO_2 <12 ml/min/kg
BNP/NT-proBNP	normal or near-normal (BNP <100 pg/ml or NT-proBNP <125pg/ml)	very high and rising (BNP >180 pg/ml or NT-proBNP >1400 pg/ml)
Echocardiography	no pericardial effusion TAPSE >2 cm	pericardial effusion TAPSE <1.5 cm
Pulmonary haemodynamics	RAP <8 mmHg CI ≥ 2.5 l/min/m^2	RAP >15 mmHg CI ≤ 2.0 l/min/m^2

6MWD: six-minute walk distance; CI: cardiac index; CPET: cardiopulmonary exercise test: RAP: right atrial pressure; RV: right ventricular; VO_2: oxygen uptake; WHO FC: World Health organisation functional class. *Depends on age, gender, height and weight.

Given the importance of accurate prognostication, a number of equations have been developed to maximise the predictive power of existing prognostic variables. The first equation was derived from a prospective registry of primary pulmonary hypertension (now classified as idiopathic, familial and anorexigen-associated PAH) initiated by the NIH in the United States before the advent of modern PAH-specific therapy in 1980s [1]. This was the first systematic epidemiological study of PAH undertaken to characterise its natural history and identify prognostic determinants. Data were collected on 194 patients treated in 32 centres across the United States between 1981 and 1985. The survival rates were poor with 68%, 48% and 34% at 1, 3 and 5 years respectively. Adverse prognostic factors included raised RAP, raised mPAP, reduced CI, WHO FC III and

IV, decreased carbon monoxide diffusing capacity (DLco) and Raynaud phenomenon. Haemodynamic variables at diagnosis were found to be most closely associated with mortality, and were used to derive an equation to estimate survival probabilities over time, P(t).

NIH equation:

$$P(t) = H(t)^{A(x,y,z)}$$
$$H(t) = 0.88 - 0.14t + 0.01t^2$$
$$A(x,y,z) = e^{(0.007325x + 0.0526y - 0.3275z)}$$

where t: number of years from diagnosis ; x: mPAP; y: RAP; z: CI.

Validation of the NIH equation was limited to one study conducted in 61 Mexican patients with primary pulmonary hypertension [43]. Variables associated with poor prognosis were elevated RAP, decreased CI, decreased S_vO_2, reduced forced vital capacity (FVC) and absence of pulmonary vasodilatory treatment. The equation was found to have a high sensitivity but a relatively low specificity for predicting survival. The overall positive predictive values were 87%, 91% and 89% at 1, 2 and 3 years respectively. The NIH equation is now obsolete, but continues to provide a benchmark for patient outcome if untreated for comparative purposes in epidemiological studies. Recently, three new prognostic equations have been developed from contemporary cohorts taking into account the effect of modern drug therapy on survival [71;72;140].

Pulmonary Hypertension Connection (PHC) Registry

The PHC Registry was initiated in 2004 to collect data on patients treated in a single practice based in Chicago United States [72]. A total of 576 incident cases of WHO group I PAH diagnosed between 1991 and 2007 were included with 247 patients in the idiopathic, familial and anorexigen-associated PAH subgroup. The observed 1-, 3- and 5-year survival rates for the whole cohort were 86%, 69% and 61% respectively. In the idiopathic, familial and anorexigen-associated PAH subgroup, the observed 1-, 3-, and 5-year survival rates were 92%, 75% and 66%

respectively, which are significantly better than those predicted using the NIH equation (65%, 43% and 32% respectively). Age, WHO FC, RAP and CI were independent predictors of survival. A new equation was developed using the same baseline haemodynamic variables and methodology as the NIH equation to predict survival in idiopathic, familial and anorexigen-associated PAH.

PHC Registry equation:

$$P(t) = e^{-A(x,y,z)t}$$

where t: number of years from diagnosis; $A(x,y,z)$: $e^{(-1.270-0.0148x+0.0402y-0.361z)}$ in calcium channel blocker (CCB) non-responders or $e^{(-3.012-0.0148x+0.0402y-0.361z)}$ in CCB responders ; x: mPAP; y: RAP; z: CI.

When the PHC Registry equation was applied to a prospective cohort of PAH patients followed up in four randomised controlled clinical trials and their extension studies, there was good agreement between predicted and observed survival [141].

French Registry

This is a prospective multicentre registry initiated by the French Network on Pulmonary Hypertension in 2002 to study the outcome of patients with WHO group I PAH during a three-year follow-up period [73]. A total of 674 consecutive patients were enrolled between 2002 and 2003, and among those 354 patients had idiopathic, familial and anorexigen-associated PAH. Survival analysis was performed in a combined analysis population including prevalent patients diagnosed within three years of enrolment and incident patients with idiopathic, familial and anorexigen-associated PAH. The 1-, 2- and 3-year survival rates for the whole PAH cohort were 87%, 76% and 67% respectively. In the combined analysis population, 1-, 2- and 3-year survival rates were 83%, 67% and 58% respectively. Female gender, 6MWD and CO were found to predict survival independently. A risk equation based on these variables was developed to estimate survival probabilities over time up to three years post-diagnosis [140].

French Registry equation:

$$P(t) = H(t)^{A(x,y,z)}$$
$$H(t) = e^{(-0.02-0.28t)}$$
$$A(x,y,z) = e^{[-(0.004x + 0.98y + 0.28z)]}$$

where t: number of years from diagnosis; x: 6MWD at diagnosis minus 280 m; y: female=1, y: male=0; z: CO at diagnosis minus 4.0 l/min

When the French Registry equation was applied to a prospective cohort of PAH patients followed up in four randomised controlled clinical trials and their extension studies, there was good agreement between predicted and observed survival [141].

Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL)

REVEAL was initiated in the United States in 2006 to study the clinical course and disease management of WHO group I PAH patients [71] and is the largest prospective multicentre PAH registry to date. Data from 2716 PAH patients treated in 54 centres were analysed to identify predictors of 1-year survival which were then used to derive a prognostic equation. One-year survival of the whole cohort from the date of enrolment was 91%. Eleven variables were found to be independently associated with mortality: aetiology, men >60 years of age, renal insufficiency, WHO FC, SBP, HR, 6MWD, BNP or NT-proBNP, pericardial effusion, % predicted DLco, RAP and PVR.

REVEAL equation:

Predicted 1-year survival: $S0(1)^{\exp(Z'\beta\gamma)}$

$S0(1) = 0.9698$ (baseline survival function)

$Z'\beta$ = linear component from the Cox model

$\gamma = 0.939$ (shrinkage factor)

The linear component is calculated by summing up coefficients for each variable or subgroup of variables (ß). The starting value is 0. It increases if ß is positive (associated with increased mortality) and decreases if negative (associated with decreased mortality) (table 1.4). Missing data are permitted in the model provided that they are randomly distributed. The REVEAL equation and its simplified 22-point risk score were found to have good discriminatory power in a prospective cohort of newly diagnosed WHO group I PAH patients [142].

Preliminary validation data show that all three equations are potentially useful prognostic tools, but their applicability in other clinical settings may be limited due to differences in study populations used for model development, recruitment periods and methodological approaches. The French Registry and PHC equations were designed to be used for patients with idiopathic, familial and anorexigen-associated PAH only whereas the REVEAL equation is applicable across all subgroups of WHO group I PAH. The PHC cohort consisted of patients recruited over a period of 16 years during which treatment strategies changed considerably with the expansion PAH-specific therapy, and this may have affected the results of survival analyses. In comparison, patient cohorts in the French and REVEAL equations were enrolled over a shorter period of time in the modern treatment era. The French Registry and PHC equations were developed to predict survival over time after diagnosis, whilst the REVEAL equation was designed to predict survival at one year after diagnosis. It is unclear if they would perform equally well in UK PH populations given the variations in patient demographics and healthcare systems across countries, and prognostic algorithms based on UK data are currently lacking.

Table 1.4. Variable coefficients for the linear component of the Cox model in the REVEAL equation [71]

WHO group I subgroup	FPAH, +0.7737	APAH-PoPH, +1.2801	APAH-CTD, +0.4624
Demographics and comorbidities	Male >60 years of age, +0.7779	Renal insufficiency, +0.6422	
WHO FC	FC I, -0.8740	FC III, +0.3454	FC IV, +1.1402
Vital signs	SBP <110 mmHg, +0.5128	Heart rate >92 bpm, +0.3322	
6MWD	6MWD ≥ 440 m, -0.5455	6MWD <165 m, +0.5210	
BNP or NT-proBNP	BNP <50 pg/ml or NT-proBNP <300 pg/ml, -0.6922	BNP >180 pg/ml or NT-proBNP >1500 pg/ml, +0.6791	
Echocardiogram	Any pericardial effusion, +0.3014		
Pulmonary function test	% predicted DLco ≥ 80%, -0.5317	% predicted DLco ≤ 32%, +0.3756	
Right heart catheterisation	Mean RAP >20 mmHg within 1 year, +0.5816	PVR >32 Wood units, +1.4062	

6MWD: six-minute walk distance; APAH: associated pulmonary hypertension; BNP: brain natriuretic peptide; CTD: connective tissue disease; DLco: carbon monoxide diffusing capacity; FPAH: familial pulmonary arterial hypertension; RAP: right atrial pressure; NT-proBNP: N-terminal pro-brain natriuretic peptide; PoPH: portopulmonary hypertension; PVR: pulmonary vascular resistance; SBP: systolic blood pressure; WHO FC: World Health Organisation functional class.

1.7 Hypotheses and aims

The aims of this thesis are to explore the use of novel non-invasive exercise variables and prognostic algorithms as alternative outcome measures to 6MWD in PH. The following hypotheses are examined in four clinical studies:

1. Non-invasive exercise variables could be used to detect treatment response in PH. These comprise:

- IGR haemodynamic measurements and isotime metabolic variables during submaximal constant-load exercise
- $P_{ET}CO_2$ during the 6MWT

2. Prognostication in PH could be improved by the use of % predicted 6MWD and a composite risk score combining key prognostic factors.

2 METHODS

2.1 The Scottish national pulmonary hypertension service

The Scottish Pulmonary Vascular Unit (SPVU) provides a tertiary PH service for the whole population in Scotland of around five millions. An epidemiology study performed in 2007 estimated the incidence of PAH in Scotland to be 7.6 cases per million per annum and the prevalence 26 cases per million [143]. A recent national audit of PH services in the UK conducted between April 2010 and March 2011 estimated the prevalence of PAH in Scotland to be 42.9 per million [144]. Patients are referred onwards to the national Pulmonary Thromboendarterectomy Service based at Papworth Hospital, Cambridge and the Cardiopulmonary Transplant Service based at Freeman Hospital, Newcastle.

Newly referred treatment-naive patients undergo a comprehensive diagnostic evaluation consisting of cardiac MRI, computed tomography, radioisotope ventilation-perfusion scan, echocardiography, lung function testing, CPET and 6MWT prior to RHC. The diagnosis of PH is based on RHC in accordance with contemporary guidelines. Prior to the publication of new consensus guidelines in August 2009, PH was defined as mPAP >25 mmHg at rest or >30 mmHg on exercise, with PCWP ≤15 mmHg and PVR >3 Wood units [145]. For patients diagnosed after August 2009, PH was defined as mPAP ≥25 mmHg at rest and was further divided into precapillary PH (PCWP ≤15 mmHg, normal or reduced CO) and postcapillary PH (PCWP >15 mmHg, normal or reduced CO) [2]). Precapillary PH consists of WHO group I PAH, WHO group III PH due to lung disease and/or hypoxia, WHO group IV CTEPH and Group V PH due to multifactorial mechanisms. Postcapillary PH consists of WHO group II PH due to heart disease. The term “PAH” is used specifically for WHO group I disease whereas “PH” is used as a general term to describe any group. Positive acute vasodilator response is defined as a drop in mPAP to <40mmHg and by >10mmHg with maintained or increased CO.

All patients diagnosed with PH receive conventional therapy including long-term warfarin, diuretics and/or supplemental oxygen. Patients with WHO group I PAH are treated with PAH-specific monotherapy (prostacyclin analogues: iloprost, treprostinil or epoprostenol; phosphodiesterase-5 inhibitors: sildenafil, tadalafil or sitaxentan [withdrawn from marketing in 2010]; endothelin receptor antagonists: bosentan or ambrisentan) and sequential combination therapy as clinically indicated. Patients with CTEPH are referred to Papworth Hospital for consideration of pulmonary thromboendarterectomy. A trial of PAH-specific therapy is offered to symptomatic CTEPH patients with inoperable distal disease, as a bridge to surgery or those with persistent PH following surgery, and selected patients with PH disproportionate to underlying heart or lung diseases or PH due to multifactorial mechanisms. Patients are followed up in the outpatient clinic every three to six months. Follow-up assessment consists of clinical evaluation, WHO FC, 6MWT, NT-proBNP and CAMPHOR score. Right heart catheterisation is not routinely repeated after initial diagnosis for monitoring purposes. Repeat CPET and cardiac MRI are carried out in selected patients as clinically indicated.

Right heart catheterisation is carried out by SPVU physicians using standard techniques. Patients breathe room air or oxygen to maintain arterial oxygen saturation (S_aO_2) above 90% in a supine position. Central venous access is obtained by inserting an 8 Fr introducer sheath in the internal jugular or femoral vein. A balloon-tipped 7 Fr Swan-Ganz catheter is passed to the RV to measure RAP, right ventricular end-diastolic pressure, mPAP and PCWP. Cardiac output is measured by TD. The final result is calculated by averaging three measurements with ≤10% variation.

PVR is calculated according to the equation:

$$\boldsymbol{PVR = (mPAP - PCWP)/CO}$$

Mixed venous blood is sampled from the main pulmonary artery to measure S_vO_2. Acute vasodilator study is carried out using inhaled nitric oxide (40 parts per

million) for 5 min to identify CCB responders. Pulmonary haemodynamics on exercise are measured using straight leg raising or supine ergometry in selected patients. Pulmonary angiogram is performed in patients with clinical evidence of CTEPH.

2.2 World Health Organisation functional class

Functional classification in PH was based on modified New York Heart Association functional classification for CHF patients [2] (Appendix 1).

2.3 N-terminal pro-brain natriuretic peptide

Venous blood samples were drawn into blood tubes containing ethylenediamine tetraacetic acid. No rest period or specific posture for venepuncture was required. Serum samples were frozen within 4 hours on the day of acquisition and stored at -80°C. Immunoassays for NT-proBNP (Roche) were subsequently performed in batches.

2.4 CAMPHOR score

CAMPHOR questionnaire was used to assess health-related patient self-reported outcome (Appendix 2) [85]. Patients were asked to answer questions in relation to their symptoms (25 items), activities (15 items) and QOL (25 items). The maximum score is 80, with higher scores indicating worse health status.

2.5 Six-minute walk test

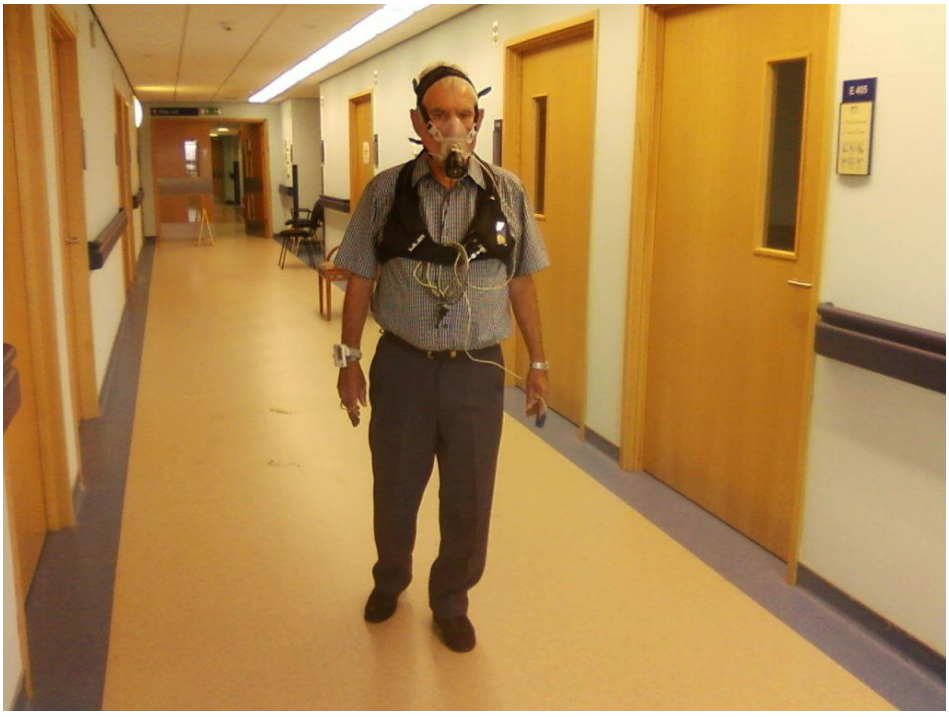
2.5.1 Test protocol

Six-minute walk test was performed by a respiratory physiologist on a 20-metre hospital corridor according to American Thoracic Society guidelines [49].

2.5.2 Telemetric metabolic measurements

Ventilatory and gas exchange variables during the 6MWT were measured using a portable metabolic device (Cortex Metamax 3B, Cranlea, Birmingham, UK) (figure 2.1). Subjects breathed through a tightly fitted face mask and carried the device (around 1 kg in weight) in a shoulder harness which allowed them to walk freely. They had previously performed a conventional 6MWT to familiarise with the test. Breath-by-breath VO_2, VCO_2, V_E, V_E/VO_2, V_E/VCO_2, VO_2/HR, $P_{ET}O_2$, $P_{ET}CO_2$, and RER were measured continuously. These data were transmitted to a laptop by telemetry and were analysed as moving averages of 8 consecutive breaths. Heart rate was recorded by a sensor strip placed around the anterior chest (Polar™) and S_aO_2 by pulse oximetry. End-6MWT values were defined as the moving average at the end of 6 min. V_E/VCO_2 slope was determined by regression analysis of V_E as a function of VCO_2.

Figure 2.1. Six-minute walk test performed using a telemetric metabolic device



The subject breathed through a tightly fitted face mask and carried the device (Cortex Metamax 3B) in a shoulder harness. The 6MWT was otherwise performed according to a standardised protocol

2.6 Incremental cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed on an electromagnetically braked cycle ergometer (Ergoline Select 200, Ergoline) under medical supervision (Dr W.T.N. Lee). Absolute contraindications included exercise-induced syncope and arrhythmias. The incremental exercise protocol consisted of rest for 2 min, followed by unloaded cycling for 3 min and then cycling at a ramp rate of 5 to 20 Watts/min to achieve peak work rate in 8 to 12 min (Bitz, Germany; Ergocard, Medisoft, Dinant, Belgium). Patients were asked to maintain a cadence rate of around 60 revolutions/min. S_aO_2 and HR were monitored continuously using pulse oximetry and 12-lead electrocardiography (ECG). Non-invasive blood pressure was measured at rest and at peak exercise. Breath-by-breath VO_2, VCO_2, V_E, ventilatory equivalent for oxygen (V_E/VO_2), V_E/VCO_2, VO_2/HR, $P_{ET}O_2$, $P_{ET}CO_2$ and RER were measured via a tightly fitted face mask and averaged over 20 s. Peak VO_2 was defined as the highest averaged value within 30 s of peak exercise. Anaerobic threshold was determined by the V-slope method. Standardised reference equations were used to calculate predicted values [41]. The development of right-to-left shunt through a PFO during exercise was assessed using a set of published pulmonary gas exchange criteria [100].

2.7 Inert gas rebreathing haemodynamic measurement

2.7.1 Principles and operational detail

Pulmonary blood flow was measured at rest and during exercise non-invasively using the IGR method (Innocor, Innovision, Odense, Denmark) [135;146;147]. Exercise was performed on a cycle ergometer (Ergoline Select 200, Ergoline, Bitz, Germany). Breath-by-breath respired gases were continuously sampled as patients breathed into a respiratory valve via a mouthpiece with the nose clipped. Gas concentrations were analysed using infrared photoacoustic gas analysers.

Prior to each IGR measurement, a 3 l Douglas bag was pre-filled with an oxygen enriched mixture containing two inert gases, N_2O and SF_6 [148]. This was obtained

by mixing a bolus from a gas bottle containing 94% oxygen, 5% N_2O and 1% SF_6 with ambient air. Under normal resting conditions, a bolus fraction of 10% and air fraction of 90% would be used to give a gas mixture of 28.3% oxygen, 0.5% N_2O and 0.1% SF_6. The volume in the rebreathing bag was adjusted continuously to accommodate for the increasing tidal volume and oxygen demand during exercise. The target volume was the highest of the following volumes: 44% of predicted vital capacity, averaged tidal volume during the preceding five breaths and the volume required to meet the maximum carbon dioxide (default 6%) and minimum oxygen limits (default 13%) in the rebreathing bag. At higher work rates, the increased oxygen demand was met by either increasing the bolus fraction or rebreathing bag volume.

IGR measurements were initiated by the operator (Dr W.T.N. Lee) at the end of expiration. At this point, the respiratory value was activated so that the patient rebreathed into the rebreathing bag in a closed circuit. The patient was instructed to empty the bag during each inspiration and breathe at a rate of at least 20 breaths/min. N_2O, being highly blood-soluble and diffusion-independent, disappeared from the alveoli into the pulmonary capillary blood at a rate proportional to PBF. SF_6, being insoluble in blood, remained in the alveoli and served to determine the final lung volume from which N_2O was removed.

The total lung volume was calculated using the following formula:

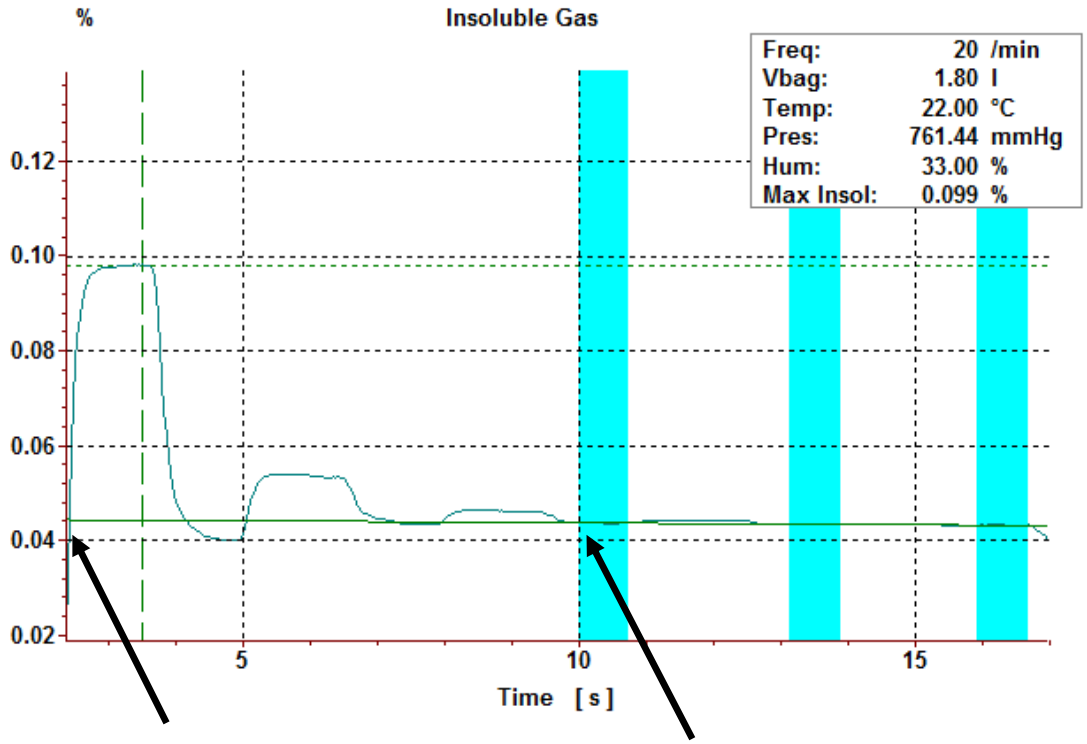
$$\boldsymbol{V_L = \frac{[SF6]_0}{[SF6]_{eq}} \cdot V_{RB}}$$

where

V_L = total systemic volume (STDP)
V_{RB} = rebreathing bag volume
$[SF_6]_0$ = initial SF_6 concentration in the rebreathing bag
$[SF_6]_{eq}$ = SF_6 concentration after good mixing (back extrapolated to time zero)

After a few breaths, SF_6 was mixed in the lungs and its concentration stabilised. The difference between maximum and minimum SF_6 concentrations within a breath was continuously analysed. Calculation of PBF would start when this difference fell below a predefined level indicating good gas mixing (set at 15% of the average of maximum and minimum SF_6 concentrations) (figure 2.2). It would usually be achieved after three breaths and calculation of PBF would take place over the subsequent three breaths. The rebreathing time should not exceed 30 seconds as re-circulation of N_2O may occur. At rest, successive measurements at rest should be performed at least 5 min apart to ensure complete washout of inert gases from the alveoli. The wash-out interval would be shorter during exercise.

Figure 2.2. SF_6 concentration during rebreathing

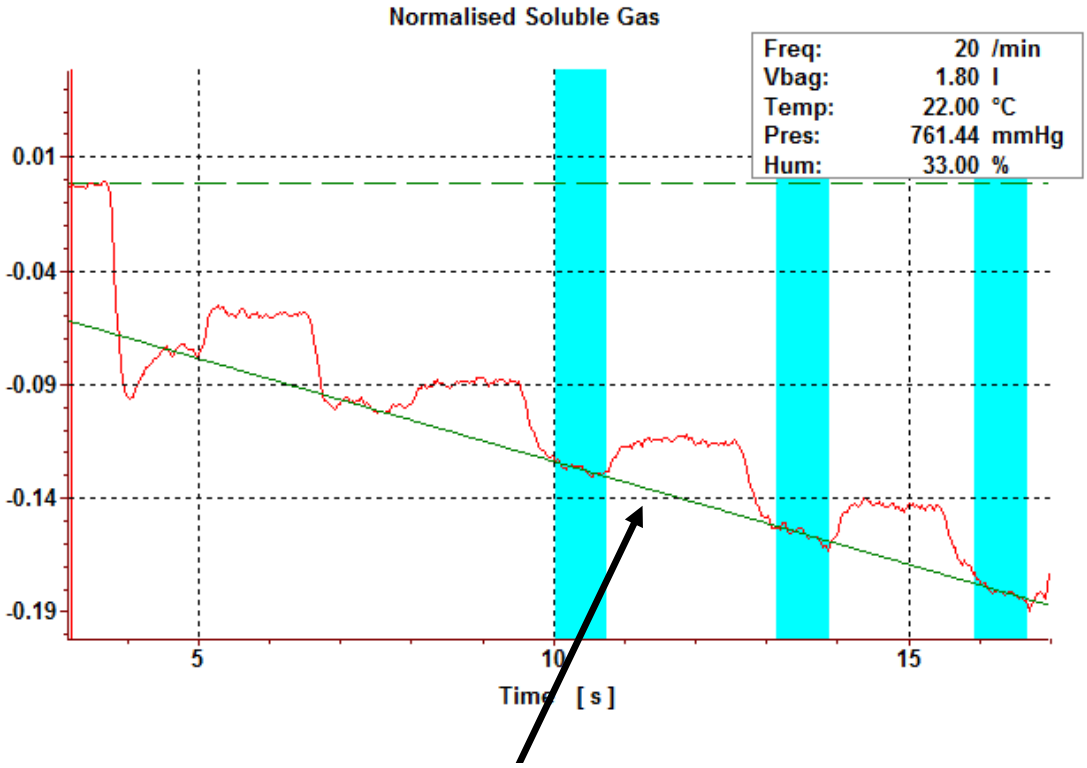


$[SF_6]_{eq}$

Good gas mixing achieved and PBF calculation starts. 3 breaths are included in the calculation indicated by the blue bars.

During the rebreathing period, the lung volume varied slightly due to changes in the rate of carbon dioxide output relative to oxygen uptake. At the start, due to slight hyperventilation, more carbon dioxide entered the alveoli whereas oxygen uptake remained constant. The lung volume increased. As the alveolar carbon dioxide concentration increased, the diffusion gradient decreased. The rate of carbon dioxide output slowed and the lung volume shrunk. Alveolar N_2O concentration was normalised for changes in lung volume using SF_6 concentration before the start of each PBF calculation. As the rate of N_2O concentration decrease was proportional to PBF and N_2O concentration itself, N_2O concentration was a mono-exponentially decreasing function of time giving rise to a linear semi-logarithmic plot of normalised N_2O against time (figure 2.3).

Figure 2.3. A semi-logarithmic plot of normalised N_2O concentration against time



Regression line through the end-expiratory points of 3 breaths after good gas mixing. The slope of the line (β) is calculated directly from the plot.

PBF was calculated using the following formula:

$$PBF = -\beta \cdot \frac{V_L \cdot C_1 + C_2}{\alpha_b}$$

where

β = slope of regression line

V_L = total systemic volume (STDP)

C_1 = 760 mmHg/(ambient pressure in mmHg – 47 mmHg)

C_2 = constant to account for absorption of N_2O into lung tissue
= Bunsen solubility coefficient of N_2O in tissue (0.407 STPD) x lung tissue volume (default 0.6 L)

α_b = Bunsen solubility coefficient of N_2O in blood (0.412 STPD)

PBF is equivalent to CO in the absence of significant intracardiac or intrapulmonary shunt. SV was derived from PBF and HR.

$$SV = \frac{PBF}{HR}$$

2.7.2 Shunt correction

The Innocor device has a built-in algorithm to correct for intrapulmonary or intracardiac shunt flow. In the algorithm, CO is derived from PBF, oxygen content in arterial blood (C_aO_2), oxygen content in pulmonary end-capillary blood (C_cO_2) and VO_2 according to the formula:

$$CO = 1/(1/PBF+(C_aO_2-C_cO_2)/VO_2)$$

where

C_aO_2 = 0.000139 x haemoglobin concentration (in g/dL) x S_aO_2

C_cO_2 = 0.000139 x haemoglobin concentration (in g/dL) x S_cO_2

S_aO_2 = arterial oxygen saturation measured by pulse oximetry

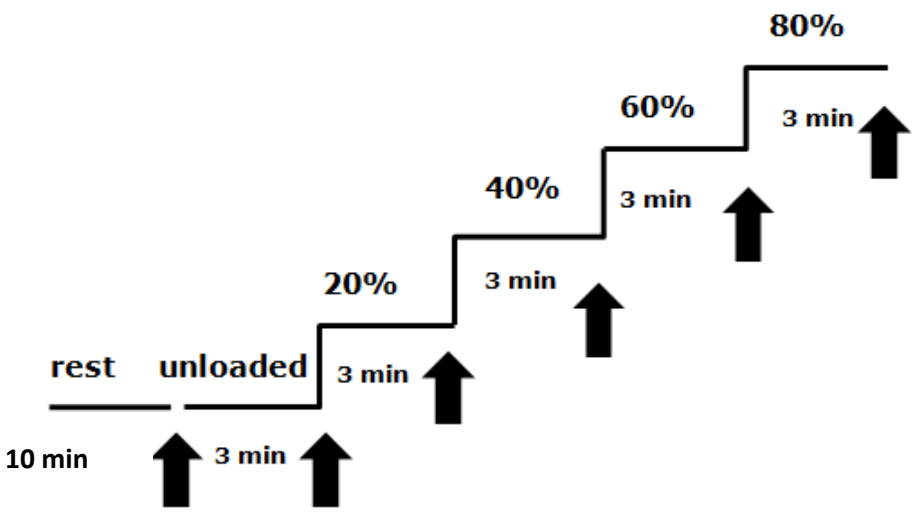
S_cO_2 = pulmonary end-capillary oxygen saturation assumed to be 98%
Haemoglobin concentration is assumed or measured by operator separately

However, the assumption that S_cO_2 reaches 98% may not apply to PH patients due to V/Q mismatch with an increase in low V/Q units combined with a low S_vO_2 as previously discussed (Chapter 1.4.1). Applying this shunt correction algorithm would overestimate the shunt flow and hence CO, especially for exercise measurements. Therefore, PBF was used instead of derived CO in the clinical studies conducted in this thesis.

2.7.3 Stepwise constant-load exercise protocol

This protocol consisted of unloaded cycling for 3 min and then cycling for 3 min successively at 20%, 40%, 60% and 80% of maximal work rate (WR_{max}) predetermined in an incremental CPET (figure 2.4). Pulmonary blood flow was measured after 10 min of rest on the cycle ergometer. Following that, PBF was measured on completion of each stage of the exercise protocol whilst cycling continued. Stroke volume was derived from PBF and HR (SV = PBF/HR). Each subject was coached on how to perform the rebreathing manoeuvre and had 2-3 practices prior to testing. Breath-by-breath metabolic data were recorded continuously during exercise except during IGR measurements each lasting for 25-30 s.

Figure 2.4. Stepwise constant-load exercise protocol

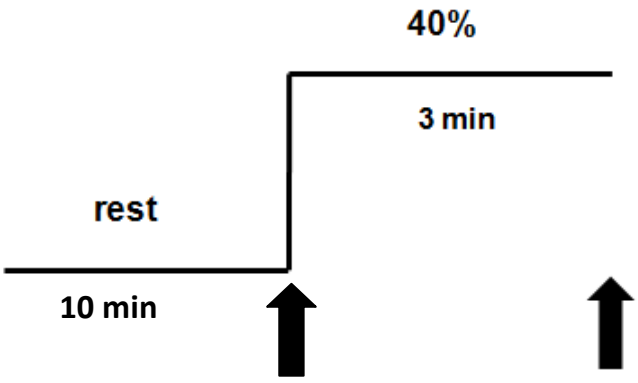


Inert gas rebreathing measurements were carried out after 10 min of rest, 3 min of unloaded cycling and 3 min of cycling successively at 20%, 40%, 60% and 80% of maximal work rate (indicated by bold arrows).

2.7.4 Submaximal constant-load exercise protocol

This protocol consisted of cycling for 3 min at 40% WR_{max} pre-determined in an incremental CPET (figure 2.5). First, successive duplicate PBF measurements were performed after 10 min of rest in the supine position, and then after 10 min of rest in the erect position on the cycle ergometer. A 5-minute interval was lapsed between duplicate measurements to allow complete washout of inert gases from the lungs. Following resting measurements, PBF was measured on completion of the exercise protocol whilst cycling continued. This was repeated after 15-20 min of rest. Stroke volume was derived from PBF and HR (SV=PBF/HR). Duplicate measurements at each stage were averaged to give the final results. Each subject was coached on how to perform the rebreathing manoeuvre and had 2-3 practices prior to testing. Breath-by-breath metabolic data were recorded continuously during exercise except during IGR measurements each lasting for 25-30 s.

Figure 2.5. Submaximal constant-load exercise protocol



Inert gas rebreathing measurements were carried out after 3 min of cycling at 40% maximal work rate (indicated by the bold arrow).

2.8 Survival analysis

2.8.1 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 or ρ >0.7) were not included. To avoid over-fitting, one covariate was included in the final model for every ten deaths. A p value <0.05 by likelihood ratio test was defined as statistically significant.

2.8.2 Kaplan Meier analysis

Kaplan Meier analysis was used to estimate survival rates using all-cause mortality as the end-point. Log-rank test/log-rank test for trends were 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.

2.8.3 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 [149]. 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. A paired or unpaired z test was used to compare the AUC of different predictor variables [150].

2.8.4 Brier score

The Brier score (BS) was used to assess the accuracy of a prognostic algorithm to predict survival. It measures the mean squared deviation of predicted probability from the actual outcome [151-153]. A BS of 0 indicates perfect prediction. A BS of 0.25 indicates that the prediction is equivalent to the outcome occurring by chance alone. A BS of 1 indicates that prediction is invariably wrong. Hence a lower BS indicates higher prediction accuracy. The performance of different prognostic algorithms was compared by determining a point estimate of the difference in BS between them and its 95% confidence interval from 200,000 bootstrap re-samples. A difference in BS of >0.02 was considered clinically relevant. A p value<0.05 was defined as statistically significant.

2.9 Patient recruitment

For the clinical studies, subjects with a diagnosis of precapillary PH confirmed at RHC were recruited at the time of diagnosis or at follow-up visits in the outpatient clinic between July 2008 and June 2010. The exclusion criteria were significant left heart dysfunction on echocardiography or lung disease (defined as forced vital capacity in one second (FEV_1) <60% predicted or FVC <60% predicted or total lung capacity (TLC) <60% predicted based on European Steel and Coal Community reference values [154]), or inability to perform exercise tests either due to absolute contraindications or disabling symptoms. All subjects received written information prior to consent.

2.10 Ethical approval

All clinical studies conducted in this thesis were reviewed and approved by the West Glasgow Research Ethics Committee. For the epidemiological studies involving retrospective collection and analysis of survival data, formal ethical review was deemed not necessary by the West Glasgow Research Ethics Committee and Papworth Hospital Research Ethics Committee.

3 INERT GAS REBREATHING HAEMODYNAMIC MEASUREMENTS IN PULMONARY HYPERTENSION

This chapter describes four studies conducted to evaluate the utility of non-invasive PBF and SV measurements by the IGR method as outcome measures in precapillary PH.

Chapter 3.1 - The relationship between work rate and IGR haemodynamic measurements during incremental exercise in precapillary PH

Chapter 3.2 - Reproducibility of IGR haemodynamic measurements in precapillary PH

Chapter 3.3 – Clinical correlates of IGR haemodynamic measurements in precapillary PH

Chapter 3.4 – Use of IGR haemodynamic measurements to detect treatment response in precapillary PH

3.1 The relationship between work rate and inert gas rebreathing haemodynamic measurements during incremental exercise in precapillary pulmonary hypertension

3.1.1 Summary

Rationale: Haemodynamic measurements are directly linked to the mechanisms of disease and influence prognosis in PH. The IGR method allows non-invasive measurement of PBF, which is equivalent to CO in the absence of significant intrapulmonary or intracardiac shunt.

Aims: The aims of the study were to evaluate the use of the IGR method to measure PBF and SV during incremental exercise, and to study their pattern of response to increasing work rate in patients with precapillary PH.

Methods: Ten healthy subjects and 10 PH patients performed stepwise constant-load exercise where they cycled for 3 min unloaded and 3 min successively at 20%, 40%, 60% and 80% of WR_{max} pre-determined in a symptom-limited incremental CPET. Pulmonary blood flow during cycling was determined using the IGR method. Stroke volume was derived from PBF and HR. Metabolic variables were simultaneously measured throughout exercise.

Results: IGR measurements at rest and during exercise were performed with ease in all subjects. Stroke volume started to plateau after unloaded cycling with maximal SV achieved at 40% WR_{max} (PH patients:68±18 ml versus healthy subjects:106±22 ml, $p<0.01$), whereas PBF rose continuously with increasing work rate in both groups. In PH patients, exercise SV tended to fall at higher work rates.

Conclusions: PBF and SV during exercise could be measured readily using the IGR method in patients with precapillary PH. Maximal SV is reached at submaximal exercise which may be used to monitor right heart function in these patients.

3.1.2 Introduction

Patients with PH have reduced SV response to exercise due to increased right ventricular afterload [155-158]. As a result, CO augmentation during exercise is blunted and is more dependent on HR rise. In healthy subjects, SV has been shown to rise with early exercise and plateau around 40%-50% of peak VO_2 whereas CO rises linearly towards peak exercise [159]. In comparison, less is known about the haemodynamic response to exercise in patients with PH. This is of clinical interest as it would provide further insights into disease severity. Previous studies using cardiac MRI [160] and electron beam computed tomography [161] showed that patients with IPAH were unable to augment SV during exercise. In these studies, exercise was performed in a supine position outside the scanner and measurements were made after exercise had ceased, therefore the true SV response to exercise may be underestimated.

Cardiac output and SV, markers of right heart function, are directly linked to the mechanisms of disease and have prognostic significance in PH. Serial measurements would be useful for monitoring disease progression and treatment response, but as they are measured invasively at RHC, repeated measurements cannot be made readily for follow-up assessment in routine clinical practice. In comparison, the IGR method allows PBF and SV to be measured non-invasively at rest and during exercise. Respired gas analysis can also be performed simultaneously to provide metabolic data on exercise response. The IGR method has been shown to be useful in the assessment of patients with CHF. The aims of this study were to explore the use of the IGR method in measuring the haemodynamic response during incremental exercise and its relationship with work rate in patients with precapillary PH.

3.1.3 Methods

Study subjects

Ten healthy subjects and 10 patients with precapillary PH participated in the study. The healthy subjects were recruited from staff members of the SPVU. They had no cardiopulmonary disease by history, physical examination, 12-lead

ECG or incremental CPET. Patients who were unable to perform CPET, receiving beta-blockers, had significant left heart dysfunction or lung disease were excluded.

Study design

The subjects first underwent an incremental CPET to determine their peak VO_2 and WR_{max} (described in Chapter 2.6). They then underwent stepwise constant-load exercise with IGR measurements within 2 weeks (described in Chapter 2.7.3).

Statistical analysis

Statistical analysis was performed using Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Data are presented as mean ± standard deviation (SD). Unpaired Student's t test was used to compare variables between healthy subjects and PH patients. Paired Student's test was used to compare variables within each group. A p value<0.05 was defined as statistically significant.

3.1.4 Results

Subject characteristics

The healthy subjects had a range of physical activity (sedentary: n=2; regular physical activity 1-3 hours per week: n=5; endurance trained: n=3). The PH group was characterised by WHO FC I (n=1), II (n=7) and III (n=2). The underlying aetiologies of PH included IPAH (n=5), CTDPAH (n=1), portopulmonary hypertension (PoPH) (n=1) and distal CTEPH (n=3). Eight patients were on stable PAH-specific therapy and two were due to start therapy. All subjects underwent incremental CPET to symptom limitation without adverse events. The PH patients had reduced WR_{max}, peak VO_2, peak VO_2/kg, peak VO_2/HR and VO_2 at AT, elevated V_E/VO_2 and V_E/VCO_2 at AT. These CPET abnormalities were characteristic of pulmonary vascular disease (table 3.1.1) [162].

Practicality of the inert gas rebreathing method

All healthy subjects and PH patients were able to perform the rebreathing manoeuvres effectively after a maximum of three practices. IGR PBF measurements were successful in all healthy subjects and 90% of occasions in PH patients. The reasons for incomplete data in the PH group were due to mask-subject interface leakage, incomplete bag emptying during the rebreathing manoeuvre and software problems leading to interruption of the exercise protocol. Overall, IGR measurements were easy to perform. The work rates at which IGR measurements were successful ranged from 28 to 260 Watts in healthy subjects and from 8 to 128 Watts in PH patients.

Table 3.1.1. Cardiopulmonary exercise characteristics

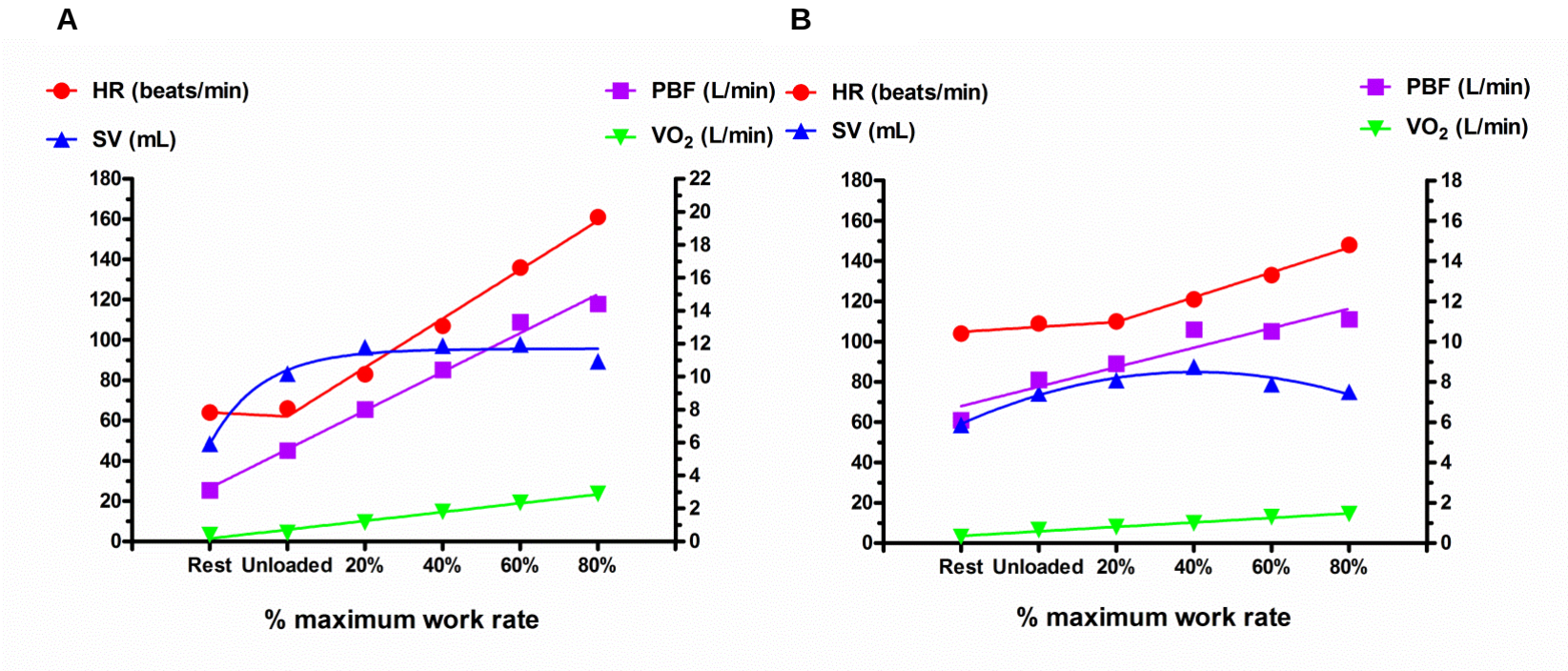
	Healthy subjects	**PH patients**
Subject, n	10	10
Age, years	34±6	49±11
Male/female, n	7/3	5/5
Peak VO_2 , l/min	2.61±0.6	1.13±0.4
Peak VO_2 , % predicted	94±14	62±30
Peak VO_2/kg, ml/min/kg	35.9±7.0	14.0±5.9
Peak WR, Watts	250±67	92±43
Peak WR, % predicted	121±24	68±39
Peak VO_2/HR, ml	14.9±3.4	8.6±2.4
Peak VO_2/HR, % predicted	100±15	78±28
VO_2 at AT, l/min	1.51±0.50	0.79±0.29
VO_2 at AT/predicted peak VO_2, %	55±15	43±20
V_E/VO_2 at AT	23±2	36±10
$V_{E/}VCO_2$ at AT	26±3	43±13

AT: anaerobic threshold; PH: pulmonary hypertension; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide; V_E/VO_2: ventilatory equivalent for oxygen ; WR: work rate.

Haemodynamic response to incremental exercise

The relationship between HR, PBF, SV and VO_2 and increasing work rate during stepwise constant-load cycle exercise in a representative healthy subject and an age- and sex-matched IPAH patient is outlined in figure 3.1.1. The IPAH patient had a higher resting HR with a smaller chronotropic response and a smaller rise in PBF and SV on exercise. These patterns were also observed when group values were compared (table 3.1.2). Pulmonary blood flow and SV were lower in PH patients than healthy subjects, at rest and at each exercise level ($p<0.01$). In both groups, SV rose on exercise with the largest increase achieved between rest and unloaded cycling. Thereafter, SV started to plateau and peaked at 40% WR_{max} whereas PBF increased continuously towards peak exercise. The mean maximal % SV rise from rest on exercise was smaller in PH patients (29±28%) compared with healthy subjects (44±30%). Stroke volume was maintained in healthy subjects at work rates higher than 40% WR_{max}. In PH patients, SV tended to fall at higher work rates, but remained above resting values (SV at 40% WR_{max}: 68±18 ml versus SV at 80% WR_{max}: 60±17 ml, $p=0.20$) (figure 3.1.2).

Figure 3.1.1. The haemodynamic response to incremental exercise in A) a healthy subject and B) an age-and sex-matched patient



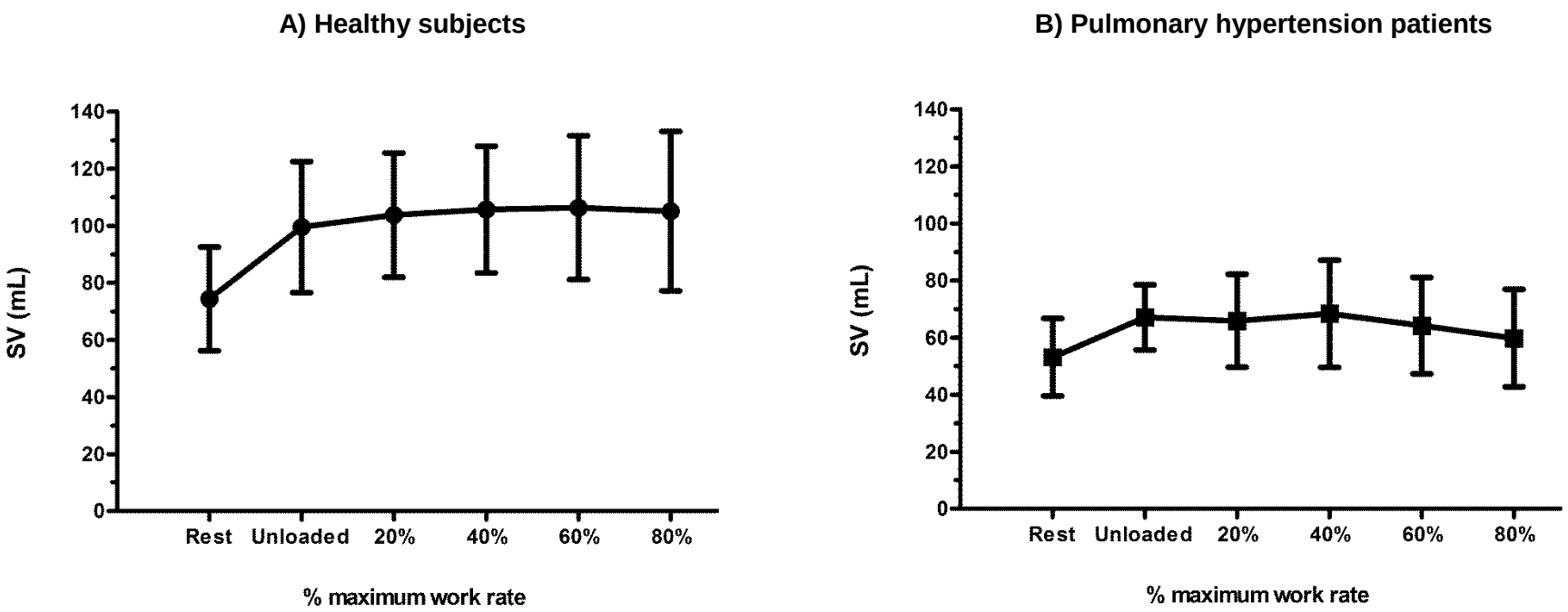
Heart rate (HR), stroke volume (SV), pulmonary blood flow (PBF) and oxygen uptake (VO_2) are plotted against exercise intensity expressed as % maximum work rate.

Table 3.1.2. Inert gas rebreathing haemodynamic measurements during incremental exercise

Study groups	Rest	Unloaded cycling	% maximal work rate			
			20%	40%	60%	80%
Healthy subjects						
PBF, l/min	5.4±1.0	7.6±1.3*	9.8±1.7*	12.4±2.3*	15±2.9*	16.9±3.5*
HR, beats/min	74±18	80±17	96±14*	119±13*	146±15*	166±12*
SV, ml	74±18	100±23*	104±22*	106±22*	106±25*	105±28*
ΔSV from rest, ml	–	+22±13	+28±16	+31±15	+29±18	+29±19
% ΔSV from rest	–	33±23	41±30	44±30	43±34	41±32
PH patients						
PBF, l/min	4.2±1.1§	6.0±1.3**§	6.2±1.5**§§	7.1±2.1**§§	7.7±2.3**§§	8.0±2.8**§§
HR, beats/min	81±12	91±11**	97±12**	106±13**	123±14**§§	140±18**§§
SV, ml	54±13§	67±11**§§	65±15**§§	68±18*§§	64±16*§§	60±17*§§
ΔSV from rest, ml	–	+11±9§	+10±8§§	+13±13§	+11±11§	+8±8§
% ΔSV from rest	–	23±20	22±14	29±28	25±19	16±14

HR: heart rate; PBF: pulmonary blood flow; SV: stroke volume; ΔSV: change in SV from rest.
**p<0.01, *p<0.05 versus rest by paired Student's test.
§§p<0.01, §p<0.05 versus healthy subjects by unpaired Student's t test.

Figure 3.1.2. The relationship between stroke volume and work rate during incremental exercise



Stroke volume (SV) is plotted against exercise intensity expressed as % maximum work rate. The data are presented as mean (symbols) and SD (error bars).

3.1.5 Discussion

This study demonstrated that PBF and SV during exercise could be readily measured using the non-invasive IGR method in patients with precapillary PH. Exercise augmentation in PBF and SV was smaller in PH patients compared to healthy subjects. In both groups, PBF increased linearly with work rate whereas maximal SV was reached at around 40% WR_{max} with the largest increase observed from rest to unloaded cycling .

As $VO_2/HR = SV \times C_{a-v}O_2$ by the Fick principle, VO_2/HR measured at CPET is considered a surrogate marker of SV, and could be used to assess SV response to exercise non-invasively. D'Alonzo et al identified an inverse relationship between VO_2/HR and PVR, and found that changes in VO_2/HR predicted changes in PVR after pulmonary vasodilator therapy [163]. However, VO_2/HR could be reduced as a result of abnormal oxygen extraction ($C_{a-v}O_2$) by exercising muscles due to myopathy. There is increasing evidence that peripheral muscle dysfunction may contribute to exercise limitation in patients with PH [164], and exercise training could significantly improve exercise capacity independent of drug treatment [165;166]. Therefore the IGR method could potentially be used to measure $C_{a-v}O_2$ non-invasively and thereby identify patients who may benefit from exercise training as an adjunct to PAH-specific therapy.

The main limitation of the study is the small sample size. As a result, it was not possible to study the haemodynamic response to exercise in subgroups stratified by WHO FC or PH aetiology. Secondly, the healthy subjects and PH patients were not matched for age or gender, but comparison of exercise response between these two groups was not the focus of this study.

3.1.6 Conclusions

Pulmonary blood flow and SV at rest and during exercise could be measured readily using the non-invasive IGR method in patients with precapillary PH. Maximal SV could be measured at submaximal exercise to give an objective assessment of right heart function independent of patient effort.

3.2 Reproducibility of inert gas rebreathing haemodynamic measurements

3.2.1 Summary

Rationale: More data on the reproducibility of IGR haemodynamic measurements are needed to evaluate their potential use as outcome measures in PH.

Aim: The aim of this study was to determine the intersession reproducibility of IGR PBF and SV measurements in healthy subjects and patients with precapillary PH.

Methods: Duplicate IGR PBF and SV measurements at rest and during submaximal constant-load exercise were obtained in ten healthy subjects and nine patients with precapillary PH on two separate days one week to one month apart. Intersession reproducibility was assessed using Bland-Altman analysis and the coefficient of variation (CoV).

Results: In healthy subjects, the mean difference between duplicate measurements of PBF was 0.0±0.9 l/min with 95% limits of agreement -1.8 to 1.9 l/min, and that of SV was 3±11 ml with 95% limits of agreement -18 to 24 ml. Median CoV was 3.8% for PBF and 4.4% for SV. In PH patients, the mean difference between duplicate measurements was 0.1±0.3 l/min for PBF and 1±4 ml for SV. Median CoV was 2.9% for PBF and 4.2% for SV.

Conclusions: IGR PBF and SV measurements are reproducible in both healthy subjects and patients with precapillary PH.

3.2.2 Introduction

In a study investigating the use of IGR method in patients with fibrotic lung disease, resting IGR measurements were found to be reproducible with a mean difference of 0.12±0.49 l/min between duplicate measurements and a CoV of 9.3% [133]. Data analysis from our unit showed that the intrasession repeatability of resting IGR SV in PH patients was good with a CoV of 6.9% [134]. However, the reproducibility of IGR measurements during exercise may differ from that at rest due to a number of factors and such data are currently lacking. The rebreathing manoeuvre is more difficult to perform during exercise especially at high work rates, and this may affect data quality. Variations in breathing patterns and changes in ventilation/perfusion matching during exercise may affect gas mixing and the uptake of inert gases from the alveoli into the pulmonary circulation. In order to explore the potential use of IGR haemodynamic measurements as outcome measures in PH, more reproducibility data are needed. The aims of this study were to determine the intersession reproducibility of IGR PBF and SV in healthy subjects and patients with precapillary PH, at rest and during exercise.

3.2.3 Methods

Study subjects

The study cohort consisted of ten healthy subjects who also took part in the study discussed in Chapter 3.1 and nine patients with precapillary PH recruited from the outpatient clinic. The exclusion criteria were inability to perform CPET, current use of beta-blockers, significant left heart dysfunction or lung disease.

Study design

The healthy subjects performed two sequential stepwise constant-load exercise tests and the PH patients performed 2 sequential submaximal constant-load exercise tests one day to one month apart. There was no change in medication or clinical status in PH patients during this interval. The exercise protocols are described in Chapters 2.7.3 and 2.7.4.

Statistical analysis

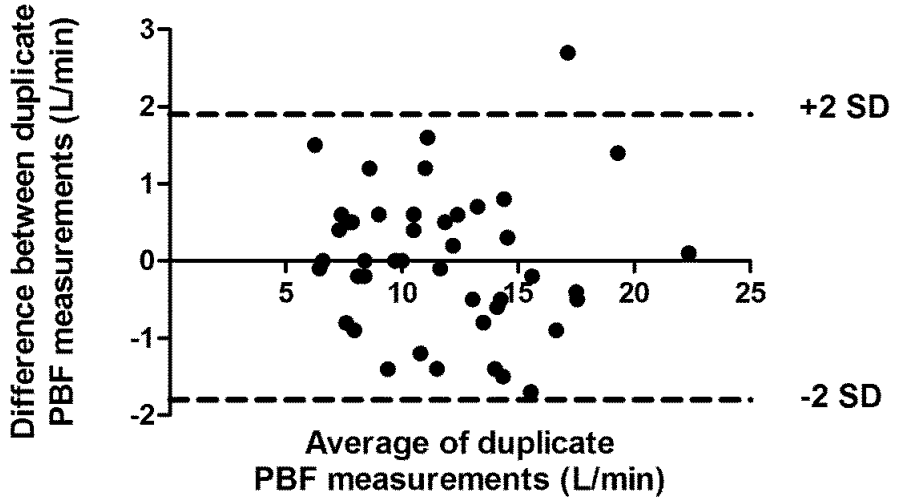
Statistical analysis was performed using Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Data are presented as median (range). Bland-Altman analysis was used to determine the mean difference between duplicate IGR measurements, SD and the 95% limits of agreement. The CoV was defined as SD of each pair of duplicate measurements as a percentage of the mean of the respective pair. The overall CoV was defined as the median CoV of the whole group. A p value<0.05 was defined as statistically significant.

3.2.4 Results

Healthy subjects

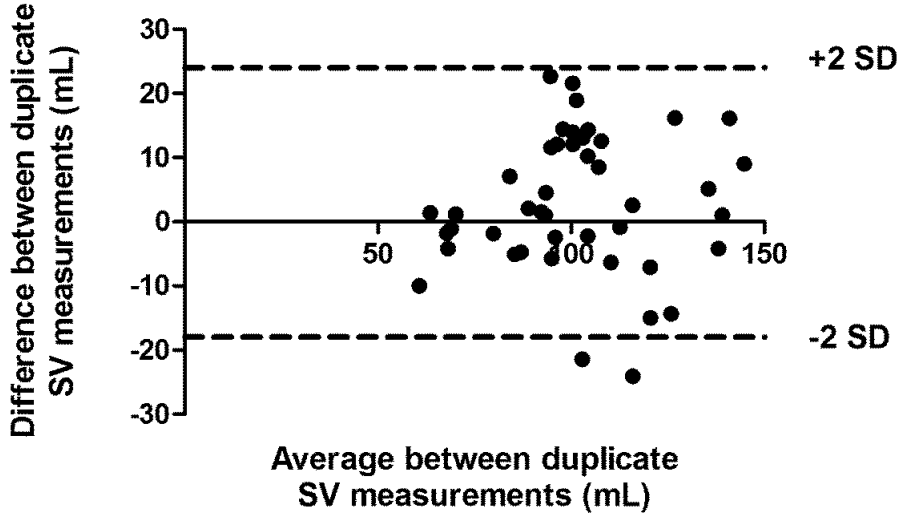
Forty four pairs of duplicate IGR PBF and SV measurements during exercise were obtained from 10 healthy subjects [female: n=3; male: n=7; age 33 (28-44)]. The mean difference between duplicate exercise PBF measurements was 0.0±0.9 l/min with 95% limits of agreement -1.8 to 1.9 l/min (figure 3.2.1). The mean difference between duplicate exercise SV measurements was 3±11 ml with 95% limits of agreement -18 to 24 ml (figure 3.2.2). Median CoV was 3.8% for PBF and 4.4% for SV.

Figure 3.2.1. Bland-Altman plot of duplicate exercise IGR PBF measurements in healthy subjects



IGR: inert gas rebreathing; PBF: pulmonary blood flow; SD: standard deviation. The dashed lines represent 95% limits of agreement.

Figure 3.2.2. Bland-Altman plot of duplicate exercise IGR SV measurements in healthy subjects



IGR: inert gas rebreathing; SD: standard deviation; SV: stroke volume. The dashed lines represent 95% limits of agreement.

Pulmonary hypertension patients

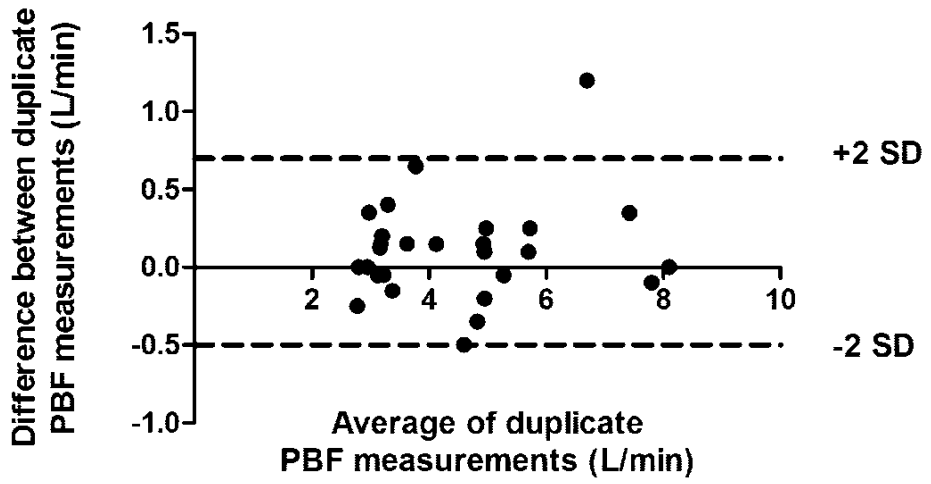
Patient characteristics are shown in table 3.2.1. Twenty seven pairs of duplicate IGR PBF and SV measurements were obtained (67% at rest, 33% during exercise). The mean difference between duplicate PBF measurements was 0.1±0.3 with 95% limits of agreement -0.5 to 0.7 l/min (figure 3.2.3). The mean difference between duplicate SV measurements was 1±4 with 95% limits of agreement -7 to 10 ml (figure 3.2.4). Median CoV was 2.9% for PBF and 4.2% for SV.

Table 3.2.1. Patient characteristics

Age, years	63 (30-79)
Female/male, n	4/5
Aetiology, n (%)	
IPAH	3 (33%)
CTEPH	6 (66%)
Exercise work rate, Watts	
Peak	60 (38-180)
40%	24 (15-72)
IGR measurements	
PBF, l/min	
supine rest	3.5 (2.8-7.3)
erect rest	3.2 (2.7-5.8)
submaximal exercise	5.3 (3.7-8.1)
SV, ml	
supine rest	57 (32-95)
erect rest	44 (30-82)
submaximal exercise	54 (30-92)

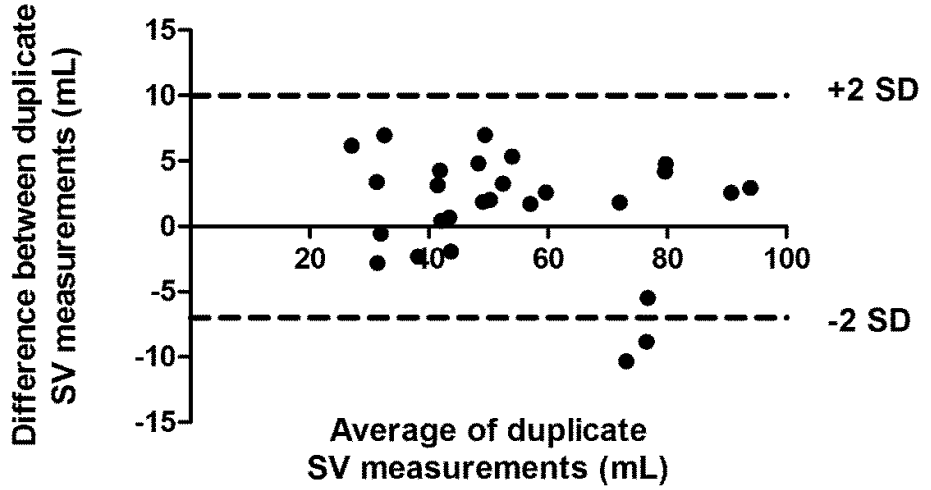
Data are represented as median (range).
CTEPH: chronic thromboembolic pulmonary hypertension; IGR: inert gas rebreathing; IPAH: idiopathic pulmonary arterial hypertension; PBF: pulmonary blood flow; SV: stroke volume. IGR measurements from the first test are shown.

Figure 3.2.3. Bland-Altman plot of duplicate IGR PBF measurements in pulmonary hypertension patients.



IGR: inert gas rebreathing; PBF: pulmonary blood flow; SD: standard deviation. The dashed lines represent 95% limits of agreement.

Figure 3.2.4. Bland-Altman plot of duplicate IGR SV measurements in pulmonary hypertension patients.



IGR: inert gas rebreathing; SD: standard deviation; SV: stroke volume. The dashed lines represent 95% limits of agreement.

3.2.5 Discussion

This study showed that the intersession reproducibility of IGR PBF and SV measurements is good in both healthy subjects and patients with precapillary PH. The reproducibility of IGR SV was poorer than that of PBF in both groups though still within acceptable limits. This may be related to the fact that SV is derived from PBF and HR, and is hence subject to more variation. The reproducibility of IGR PBF and SV is maintained during exercise in healthy subjects, but it was not possible to make a direct comparison between resting state and exercise in PH patients as the number of duplicate exercise measurements was relatively small. However, based on our data analysis, it would be reasonable to assume that the reproducibility of IGR PBF and SV during exercise in PH patients is comparable to that at rest.

3.2.6 Conclusions

IGR PBF and SV measurements demonstrate good intersession reproducibility in both healthy subjects and patients with precapillary PH.

3.3 Clinical correlates of inert gas rebreathing haemodynamic measurements

3.3.1 Summary

Rationale: IGR PBF and SV at rest and during exercise are direct measures of right heart function, and should correlate with other established markers of disease of severity in PH.

Aim: The aim of this study was to determine the clinical correlates of IGR PBF and SV in PH.

Methods: Data on patient demographics, RHC, lung function, WHO FC, NT-proBNP, CAMPHOR, incremental CPET variables and 6MWD were collected in 42 patients with precapillary PH. Correlations between these variables and IGR PBF and SV, measured at rest and at submaximal exercise (40% WR_{max}), were determined by Pearson or Spearman correlation coefficients depending on data distribution.

Results: IGR measurements at supine rest correlated with 6MWD, WHO FC, NT-proBNP, CAMPHOR (except PBF), CPET variables (peak VO_2, peak VO_2/HR, V_E/VCO_2 at AT, $P_{ET}CO_2$ at AT) and RHC variables (RAP, mPAP, CO by TD, SV by TD, PVR and S_vO_2). IGR measurements at erect rest correlated with 6MWD, WHO FC (except PBF), NT-proBNP, CAMPHOR (except PBF), CPET variables (peak VO_2, peak VO_2/HR, V_E/VCO_2 at AT, $P_{ET}CO_2$ at AT) and RHC variables (CO by TD, SV by TD and PVR). IGR measurements at submaximal exercise correlated with 6MWD, WHO FC (except PBF), NT-proBNP, CAMPHOR, CPET variables (peak VO_2, peak VO_2/HR, V_E/VCO_2 at AT, $P_{ET}CO_2$ at AT) and RHC variables (mPAP (except PBF), CO by TD, SV by TD, PVR and S_vO_2). There was no significant correlation between % predicted DLco and IGR measurements at rest or at submaximal exercise.

Conclusions: Resting and submaximal exercise IGR PBF and SV correlate with other conventional markers of disease severity in PH and have the potential to be used as alternative outcome measures.

3.3.2 Introduction

A number of clinical, exercise, haemodynamic and biological parameters have established prognostic importance and are widely used to guide clinical management in PH [2]. These include WHO FC, 6MWD, peak VO_2, RHC variables and biomarkers such as NT-proBNP. Among them, the most relevant prognosticator is 6MWD which has been used as the primary end-point in most clinical trials of currently approved PAH-specific therapy. The efficacy of any new pharmacological agents will also be judged on their ability to improve 6MWD. Other potential outcome measures include CAMPHOR, the first health-status assessment tool developed specifically for PH patients [85] and % predicted DLco which was shown to predict mortality independently of age, lung parenchymal abnormalities on computed tomography, WHO FC and haemodynamic variables in a large cohort of patients with WHO group I PAH [167]. IGR PBF and SV are direct measures of right heart function and hence closely linked to the mechanisms of disease in PH. The aim of this study was to determine the clinical correlates of IGR PBF and SV, at rest and during submaximal exercise.

3.3.3 Methods

Study subjects

42 patients with precapillary PH who were either newly diagnosed or on stable PAH-specific therapy over a period of at least 6 months were included. The exclusion criteria were inability to perform exercise tests, current use of beta-blockers, significant left heart dysfunction or lung disease.

Study design

Study patients first underwent a symptom-limited incremental CPET. On a separate day within 2 weeks, they performed a 6MWT (described in Chapter 2.5.1) and a submaximal constant-load CPET with IGR measurements (described in Chapter 2.7.4) on the same day at least 30 min apart and in this order. IGR PBF and SV were measured at supine rest, erect rest and after 3 min of cycling at 40% WR_{max} predetermined in the incremental CPET. Data on demographics, lung function, incremental CPET, RHC, WHO FC, NT-proBNP, CAMPHOR were collected at diagnosis for newly diagnosed patients, and within three months for stable patients (except RHC and lung function as these tests were not routinely repeated during follow-up). For newly diagnosed patients, exercise tests were performed after RHC prior to initiation of PAH-specific therapy (mean 2.1 weeks, range 1 day to 5.4 weeks).

Statistical analysis

Statistical analysis was performed using Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Continuous variables were tested for normality using D'Agostino and Pearson omnibus normality test. Normally distributed variables are shown as mean ± SD and non-normally distributed variables are shown as median (interquartile range, IQR). Categorical variables are presented as number (%). Correlations between demographics, RHC, lung function, WHO FC, NT-proBNP, CAMPHOR, incremental CPET variables, 6MWD and IGR PBF and SV, measured at rest and during submaximal exercise, respectively, was determined by Pearson correlation coefficient (r) if both variables were normally distributed or otherwise by Spearman correlation coefficient (ρ). A p value<0.05 was defined as statistically significant.

3.3.4 Results

Patient characteristics are shown in tables 3.3.1.and 3.3.2

Correlates of resting IGR measurements

IGR measurements at supine rest correlated with 6MWD, WHO FC, NT-proBNP, CAMPHOR (except PBF), incremental CPET variables (peak VO_2, peak VO_2/HR, V_E/VCO_2 at AT and $P_{ET}CO_2$ at AT) and RHC variables (mRAP, mPAP, CO by TD, SV by TD, PVR and S_vO_2). IGR measurements at erect rest correlated with 6MWD, WHO FC (except PBF), NT-proBNP, CAMPHOR score (except PBF), incremental CPET variables (peak VO_2, peak VO_2/HR, V_E/VCO_2 at AT and $P_{ET}CO_2$ at AT) and RHC variables (CO by TD, SV by TD and PVR) (table 3.3.3). There was no correlation between lung function and IGR PBF or SV.

Correlates of submaximal exercise IGR measurements

IGR measurements during submaximal exercise correlated with 6MWD, WHO FC (except PBF), NT-proBNP, CAMPHOR, incremental CPET variables (peak VO_2, peak VO_2/HR, V_E/VCO_2 at AT and $P_{ET}CO_2$ at AT) and RHC variables (mPAP (except PBF), CO by TD, SV by TD, PVR and S_vO_2) (table 3.3.4). There was no correlation between lung function (% predicted FEV_1, FVC or DLco) and IGR PBF or SV.

Table 3.3.1. Characteristics of the whole cohort

Age, years	57±15
Gender, n (%)	
Female	19 (45)
Male	23 (55)
Aetiology, n (%)	
IPAH	20 (46)
CTDPAH	5 (12)
PoPH	2 (5)
CTEPH	14 (33)
sarcoidosis	1 (2)
6MWD, m	414±112
WHO FC, n (%)	
I	1 (2)
II	24 (57)
III	17 (40)
NT-proBNP, pg/ml	239 (88-1602)
CAMPHOR	25±20
Incremental CPET variables	
Peak VO_2, l/min	0.891 (0.764-1.20)
Peak VO_2/HR, ml	7.6±2.2
V_E/VCO_2 at AT	48 (41-53)
$P_{ET}CO_2$ at AT, mmHg	27±6
IGR measurements	
supine rest	
PBF, l/min	4.1±1.1
SV, ml	56±20
erect rest	
PBF, l/min	3.5±0.9
SV, ml	45±14
submaximal exercise	
PBF, l/min	5.6±2.0
SV, ml	53±20

Data are presented as mean ± SD or median (IQR). 6MWD: six-minute walk distance; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; CPET: cardiopulmonary exercise test; CTDPAH: connective tissue disease associated pulmonary arterial hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; IGR: inert gas rebreathing; IPAH: idiopathic pulmonary arterial hypertension; PBF: pulmonary blood flow; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; PoPH: portopulmonary hypertension; NT-proBNP: N-terminal pro-brain natriuretic peptide; SV: stroke volume; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2 at AT: ventilatory equivalent for carbon dioxide at anaerobic threshold; WHO FC: World Health Organisation functional class.

Table 3.3.2. Characteristics of newly diagnosed patients

Patient number, n	26
RHC haemodynamics	
RAP, mmHg	7 (5-10)
mPAP, mmHg	47±13
CO by TD, l/min	4.3±1.6
SV by TD, ml	61±28
PVR, Wood units	8.4 (6.1-16.0)
$S_vO_{2,}$ %	66±11
Lung function, % predicted	
FEV_1	84±17
FVC	101 (84-107)
DLco	54±21

Data are presented as mean ± SD or median (IQR).
CO: cardiac output; DLco: diffusing capacity for carbon dioxide; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; RAP: right atrial pressure; RHC: right heart catheterisation; S_vO_2: mixed venous saturation; SV: stroke volume; TD: thermodilution.

Table 3.3.3. Correlates of IGR measurements

	PBF_{sr}	SV_{sr}	PBF_{er}	SV_{er}	PBF_{ex}	SV_{ex}
6MWD	r=0.42, p<0.01	r=0.42, p<0.001	r=0.41, p<0.01	r=0.42, p<0.01	r=0.56, p<0.01	r=0.55, p<0.01
WHO FC	r=-0.47, p<0.01	r=-0.50, p<0.01	r=-0.25, p=0.12	r=-0.45, p<0.01	r=-0.29, p=0.07	r=-0.41, p<0.01
NT-proBNP	ρ=-0.65,p<0.01	ρ=-0.71, p<0.01	ρ=-0.49, p<0.05	ρ=-0.50, p<0.01	ρ=-0.78, p<0.01	ρ=-0.74, p<0.01
CAMPHOR	r=-0.26, p=0.14	r=-0.40, p<0.05	r=-0.29, p=0.06	r=-0.43, p<0.01	r=-0.42, p<0.01	r=-0.50, p<0.01
Peak VO_2	ρ =0.58, p<0.01	ρ=0.63, p<0.01	ρ=0.56, p<0.01	ρ=0.56, p<0.01	ρ=0.78, p<0.01	ρ=0.77, p<0.01
Peak VO_2/HR	r=0.57, p<0.01	r=0.66, p<0.01	r=0.51, p<0.01	r=0.62, p<0.01	r=0.72, p<0.01	r=0.82, p<0.01
V_E/VCO_2 at AT	ρ=-0.64, p<0.01	ρ=-0.67, p<0.01	ρ=-0.60, p<0.01	ρ=-0.62, p<0.01	ρ=-0.65, p<0.01	ρ=-0.72, p<0.01
$P_{ET}CO_2$ at AT	r=0.60, p<0.01	r=0.58, p<0.01	r=0.60, p<0.01	r=0.62, p<0.01	r=0.64, p<0.01	r=0.71, p<0.01

r: Pearson correlation coefficient; ρ: Spearman correlation coefficient.
6MWD: six-minute walk distance; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; IGR: inert gas rebreathing; NT-proBNP: N-terminal pro-brain natriuretic peptide; PBF_{er}: pulmonary blood flow at erect rest; PBF_{ex}: pulmonary blood flow at exercise; PBF_{sr}: pulmonary blood flow at supine rest; $P_{ET}CO_2$ at AT: end-tidal carbon dioxide partial pressure at anaerobic threshold; SV_{er}: stroke volume at erect rest; SV_{ex}: stroke volume at exercise; SV_{sr}: stroke volume at supine rest; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2 at AT: ventilatory equivalent for carbon dioxide at anaerobic threshold; WHO FC: World Health Organisation functional class.

Table 3.3.4. Correlates of IGR measurements (continued)

	PBF_{sr}	SV_{sr}	PBF_{er}	SV_{er}	PBF_{ex}	SV_{ex}
RAP	ρ=-0.45, p=0.03	ρ=-0.50, p=0.01	ρ=-0.20, p=0.35	ρ=-0.29, p=0.16	ρ=-0.25, p=0.21	ρ=-0.35, p=0.09
mPAP	r=-0.42, p=0.04	r=-0.50, p=0.01	r=-0.01, p=0.97	r=-0.25, p=0.22	r=-0.39, p=0.05	r=-0.47, p=0.02
CO by TD	r=0.70, p<0.01	r=0.63, p<0.01	r=0.57, p<0.01	r=0.51, p=0.01	r=0.79, p<0.01	r=0.71, p<0.01
SV by TD	r=0.79, p<0.01	r=0.80, p<0.01	r=0.58, p<0.01	r=0.67, p<0.01	r=0.85, p<0.01	r=0.83, p<0.01
PVR	ρ=-0.78, p<0.01	ρ=-0.79, p<0.01	ρ=-0.48, p=0.02	ρ=-0.51, p<0.01	ρ=-0.82, p<0.01	ρ=-0.82, p<0.01
S_vO_2	r=0.57, p<0.01	r=0.51, p=0.01	r=0.28, p=0.18	r=0.27, p=0.20	r=0.61, p<0.01	r=0.55, p<0.01
% predicted DLco	r=0.01, p=0.97	r=0.03 p=0.88	r=0.12, p=0.59	r=0.02, p=0.92	r=0.23, p=0.27	r=0.12, p=0.58

r: Pearson correlation coefficient; ρ: Spearman correlation coefficient.
CO: cardiac output; DLco: diffusing capacity for carbon dioxide; mPAP: mean pulmonary artery pressure; PBF_{er}: pulmonary blood flow at erect rest; PBF_{ex}: pulmonary blood flow at exercise; PBF_{sr}: pulmonary blood flow at supine rest; PVR: pulmonary vascular resistance; RAP: right atrial pressure; S_vO_2: mixed venous saturation; SV_{er}: stroke volume at erect rest; SV_{ex}: stroke volume at exercise; SV_{sr}: stroke volume at supine rest; TD: thermodilution.

3.3.5 Discussion

Data analysis from this study showed that in patients with precapillary PH, IGR PBF and SV measured at supine rest, erect rest and submaximal exercise (40% WR_{max}) correlate with other markers of disease severity including 6MWD, WHO FC, NT-proBNP, CAMPHOR, CPET variables and haemodynamics measured at RHC, but there was no correlation with lung function.

Correlations between 6MWD and IGR measurements were stronger during submaximal exercise than at rest but overall only modest. Limitations of the 6MWT may explain the relatively loose relationships. Performance at the 6MWT can be influenced by many factors unrelated to PH such as coexisting cardiopulmonary, neurological and musculoskeletal abnormalities, subject volition and walking efficiency. It is also subject to a ceiling effect in its ability to detect clinical change in fitter patients [70;93]. On the other hand, IGR PBF and SV are direct measures of right heart function and hence closely linked to disease severity. They are not affected by an ceiling effect or subject volition if exercise is performed at standardised submaximal work rates. There were strong correlations between peak VO_2 (r=0.76-0.80) and submaximal exercise IGR measurements. This indicates that IGR PBF or SV measured at 40% WR_{max} could be used as surrogate markers of peak exercise capacity and this would provide an objective assessment independent of patient effort.

3.3.6 Conclusions

Resting and submaximal exercise IGR PBF and SV correlate with other conventional markers of disease severity and may be used as alternative outcome measures in PH.

3.4 Use of inert gas rebreathing haemodynamic measurements to detect treatment response in precapillary pulmonary hypertension

3.4.1 Summary

Rationale: Haemodynamic measurements may be superior to 6MWD as outcome measures in PH as they are directly linked to the mechanisms of disease and are not subject to a ceiling effect. The aim of this study was to determine if treatment response in precapillary PH could be detected by PBF and SV measured non-invasively using the IGR method at rest and during submaximal constant-load exercise.

Methods: Twenty four patients with precapillary PH receiving de novo or modified PAH-specific therapy were studied. Isotime metabolic variables, IGR PBF and SV were measured at rest and during submaximal constant-load exercise at 40% WR_{max} alongside conventional outcome measures, at baseline and after 3 months of new therapy.

Results: At follow-up there was a significant increase in IGR PBF (supine rest: mean 0.7±0.9 l/min, erect rest: 0.7±0.8 l/min, exercise: 0.8±1.0 l/min, $p<0.005$) and SV (supine rest: 7±10 ml, erect rest: 10±11 ml, exercise: median 6 (IQR 3-11) ml, $p<0.005$). There was a trend for 6MWD to increase by 17±42 m or 29 (13-47) m ($p=0.061$), whereas WHO FC, NT-proBNP or CAMPHOR score were unchanged. In patients with higher baseline 6MWD, IGR measurements were more sensitive than 6MWD in detecting treatment response.

Conclusions: Non-invasive IGR haemodynamic measurements could be used to detect treatment response in patients with precapillary PH and may be more responsive to change than 6MWD in fitter patients.

3.4.2 Introduction

An impaired exercise SV and consequently CO response is the fundamental pathophysiological consequence of PAH [15]. PAH-specific therapy is aimed at reducing PVR and right ventricular afterload, thereby improving CO response to exercise. Measurement of CO at RHC would allow direct monitoring of disease progression and treatment response, but serial RHC is inconvenient in clinical practice owing to its invasive nature. As a result, 6MWD, a measure of functional exercise capacity, has been used as a simple surrogate marker of exercise CO, and has been established as the primary end-point in most clinical trials of PAH therapy [89]. However, several factors exist which may compromise the reliability of this test as an outcome measure, such as subject volition, comorbid respiratory and musculoskeletal abnormalities, and a ceiling effect in its ability to detect clinical change in fitter patients [70;93]. Additionally, it is subject to a learning effect [168].

Measurement of CO by the IGR method could be an alternative outcome measure to 6MWD. It is a direct measurement of haemodynamics and is hence disease-specific. Cardiac output is not subject to a ceiling effect due to its linear relationship with peak VO_2 and so, when combined with exercise, may be more sensitive than 6MWD in detecting early change in fitter patients [78]. As SV reaches its maximum at around 40-50% maximal work rate [169], maximal SV could be measured during submaximal constant-load exercise to give an objective measurement independent of patient effort. This form of exercise would also allow isotime comparison of metabolic variables which were shown to be more sensitive than variables measured at peak exercise in demonstrating improved exercise capacity from therapeutic interventions in COPD [112;113;170]. Cardiac output measured non-invasively by the IGR method has been validated against the TD and Fick methods in patients with PH and interstitial lung disease [132-134].

The aims of this study were to determine if response to PAH-specific therapy in patients with precapillary PH could be detected by changes in IGR haemodynamic measurements and also by isotime comparison of metabolic

indices of cardiac function (VO_2 and VO_2/HR) and ventilatory efficiency (V_E/VCO_2 and $P_{ET}CO_2$), at rest and during submaximal constant-load exercise.

3.4.3 Methods

Study subjects

The inclusion criteria were incident patients diagnosed with precapillary PH including WHO group I PAH (except CHD) and CTEPH started on de novo PAH-specific therapy, and prevalent patients in whom PAH-specific therapy was modified. Patients who were unable to perform a 6MWT or CPET were excluded.

Study design

Study patients underwent a symptom-limited incremental CPET and a 6MWT at baseline (described in Chapters 2.6 and 2.5.1 respectively). On a separate day within two weeks they performed a second 6MWT using a telemetric metabolic device (described in Chapter 2.5.2) and submaximal constant-load CPET with IGR measurements on the same day 30 min apart and in this order. These two tests were repeated after 3 months of new PAH-specific therapy. In addition, conventional outcome measures including WHO FC, NT-proBNP and CAMPHOR score were recorded at baseline and follow-up.

IGR and isotime metabolic measurements during submaximal constant-load exercise

The IGR method is described in Chapter 2.7.4. Duplicate PBF measurements were made after 10 min of rest in the supine position and again after 10 min of rest on the upright cycle ergometer. There was an interval of 5 min between duplicate PBF measurements to ensure complete washout of inert gases from the lungs. The patient then underwent a constant-load CPET. After 3 min of cycling, PBF was measured while the patient continued to cycle. S_aO_2 and HR were measured continuously by pulse oximetry and 12-lead ECG. Breath-by-breath respired air was collected via a mouthpiece with the nose clipped and moving averages of metabolic data over eight breaths were obtained. End-exercise values were defined as the highest of the last eight averaged values

and were used for isotime analysis at baseline and follow-up. Isotime analysis refers to comparison of measurements made at standardised time with an identical workload history. Stroke volume was derived from PBF and HR (PBF divided by HR). $C_{a-v}O_2$ was calculated from VO_2 and PBF (VO_2 divided by PBF). The exercise protocol was repeated after 15-20 min of rest. Duplicate measurements in each step were averaged to give the final results. The work rate remained the same for submaximal constant-load exercise at follow-up.

Statistical analysis

Statistical analysis was performed using Statview version 5.0.1 (SAS Institute, Cary, NC, USA) and Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Assuming a treatment-induced change in 6MWD of 40 m and a SD of 54 m based on data from a group of patients with IPAH receiving PAH-specific therapy [44], the sample size required to detect a treatment effect with 80% power and 5% two-tailed level of significance by paired t test would be 17. A post-hoc power calculation was performed using data observed in this study. Continuous variables were tested for normality using D'Agostino and Pearson omnibus normality test. Normally distributed variables are shown as mean ± SD and non-normally distributed variables are shown as median (IQR). Categorical variables are presented as number (%). Comparison between baseline and follow-up 6MWD, NT-proBNP, CAMPHOR, IGR and isotime metabolic measurements were made by paired t test or Wilcoxon signed rank test depending on data distribution. Comparison between baseline and follow-up WHO FC was made by Chi-squared test. Correlation between two variables that were both normally distributed was determined by Pearson correlation coefficient (r), and otherwise by Spearman correlation coefficient (ρ). Post-hoc analysis was carried out by stratifying patients into high and low 6MWD groups using the median baseline 6MWD. Comparison of baseline characteristics between 6MWD subgroups was made by unpaired t test or Mann-Whitney U test depending on data distribution. A p value <0.05 was defined as statistically significant.

3.4.4 Results

Patient characteristics

Twenty four patients were recruited and their characteristics are shown in table 3.4.1. Incremental CPET abnormalities were consistent with moderately severe exercise impairment due to pulmonary vascular disease (table 3.4.2). Other factors such as the use of digoxin, beta-blockers and diuretics were unchanged during the study period. None of the study patients were involved in exercise programmes or had evidence of exercise induced right-to-left shunt. The mean time interval between baseline testing and follow-up assessment was 3.3 months (range 2.4–4.5).

Table 3.4.1. Patient Characteristics

Age, years	59±15
Female, n (%)	8 (33)
Aetiology, n (%)	
IPAH	12 (50)
CTDPAH	2 (8)
PoPH	1 (4)
CTEPH	8 (33)
Sarcoidosis	1 (4)
Baseline haemodynamics	
RAP, mmHg	7±5
mPAP, mmHg	48±13
CO, l/min	3.9 (3.3-5.3)
CI, l/min/m^2	2.2±0.7
PVR, Wood units	10.6±5.7
S_vO_2, %	65±11
De novo monotherapy (n=22), n (%)	
Intravenous epoprostenol	1 (4)
Sildenafil	13 (54)
Bosentan	6 (25)
Ambrisentan	2 (8)
Combination therapy (n=1), n (%)	
Nebulised iloprost added to sildenafil	1 (4)
Agent change (n=1), n (%)	
Bosentan changed to sildenafil	1 (4)

Data are expressed as mean ± SD, median (IQR) or n (%).
CI: cardiac index; CO: cardiac output; CTDPAH: connective tissue disease associated pulmonary arterial hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; mPAP: mean pulmonary artery pressure; PoPH: portopulmonary hypertension; PVR: pulmonary vascular resistance; RAP: right atrial pressure; S_vO_2: mixed venous saturation.

Table 3.4.2. Incremental cardiopulmonary exercise test characteristics

Peak VO_2, l/min	0.971±0.355
Peak VO_2/kg , ml/min/kg	12±4
Peak VO_2, % predicted	55±19
Peak work rate, Watts	61 (47-97)
Peak work rate, % predicted	56±23
VO_2 at AT, l/min	0.694±0.173
VO_2 at AT, % predicted peak VO_2	42±11
Peak HR, beats/min	132±27
Peak HR, % predicted	85±12
Peak VO_2/HR, ml	7±2
Peak VO_2/HR, % predicted	70±24
Peak V_E, l/min	62.4 (52.6-81.1)
V_E/VO_2 at AT	48 (40-56)
V_E/VCO_2 at AT	51 (47-64)
$P_{ET}CO_2$ at AT, mmHg	24±5
End-exercise $P_{ET}CO_2$, mmHg	20±5
Resting S_aO_2, %	95±3
End-exercise S_aO_2, %	90±6
RER	1.16±0.15

Data are expressed as mean ± SD or median (IQR).
AT: anaerobic threshold; HR: heart rate; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; RER: respiratory exchange ratio; S_aO_2: arterial oxygen saturation; V_E: minute ventilation; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide; V_E/VO_2: ventilatory equivalent for oxygen.

Conventional outcome measures

After 3 months of new therapy, 6MWD increased from 384±115 m to 401±111 m (mean change 17±42 m; median change 29 (3-47) m, p=0.061). There were no significant changes in WHO FC (baseline: 50% in FC II and 50% in FC III; follow-up: 8% in FC I, 54% in II and 38% in FC III, p=0.215), NT-proBNP (baseline: 1145±1319 pg/ml; follow-up: 1014±1066 pg/ml, p=0.436) or CAMPHOR score (baseline: 33±21; follow-up: 30±21, p=0.435).

IGR and isotime metabolic measurements

After 3 months of new therapy, IGR measurements at supine rest, erect rest and submaximal exercise increased significantly at follow-up (table 3.4.3). The change in PBF with therapy was 0.7±0.9 l/min at supine rest, 0.7±0.8 l/min at erect rest and 0.8±1.0 l/min at submaximal exercise. The change in SV with therapy was 7±10 ml at supine rest, 10±11 ml at erect rest and 6 (3-11) ml at submaximal exercise. Isotime VO_2 increased by 0.025±0.042 l/min (p=0.010) and VO_2/kg by 0.3±0.6 ml/min/kg (p=0.012). $C_{a-v}O_2$ decreased by 1.6±3.0 ml/dl (p=0.021) and RER by 0.02±0.05 (p=0.025). There were no significant changes in VO_2/HR, V_E/VCO_2 or $P_{ET}CO_2$ (table 3.4.4). There were modest correlations between therapy-induced changes in IGR and isotime metabolic measurements during constant-load exercise (figures 3.4.1 and 3.4.2). The mean or median % change in IGR measurements from baseline was greater than that for 6MWD (figure 3.4.3).

In the post-hoc analysis the high 6MWD group (477±80 m) were significantly younger and had less severe exercise impairment on incremental CPET than the low 6MWD group (291±47 m) (table 3.4.5). Six-minute walk distance improved by 34±25 m (p<0.001) and isotime VO_2 by 0.033±0.031 l/min (p=0.009) with new therapy in the low 6MWD group whereas neither variable changed significantly in the high 6MWD group. In comparison, there remained a significant improvement in IGR measurements in both groups (table 3.4.6). Stratified isotime metabolic data are shown in table 3.4.7

Table 3.4.3. IGR measurements during submaximal constant-load exercise

	Baseline	Follow-up	p value
HR, beats/min			
Supine rest	81±15	79±11	0.519
Erect rest	84±16	80±11	0.155
Exercise	114±18	112±16	0.274
Exercise response*	31±9	33±11	0.408
PBF, l/min			
Supine rest	4.0±1.0	4.7±1.4	0.002
Erect rest	3.2±0.6	3.9±0.9	<0.001
Exercise	5.3±1.7	5.7 (4.1-8.1)	0.003
Exercise response*	2.1±1.5	2.1±1.6	0.921
SV, ml			
Supine rest	53±22	58 (44-68)	0.002
Erect rest	40±13	50±15	<0.001
Exercise	48±18	56±21	0.004
Exercise response*	8±12	5±13	0.176

Data are expressed as mean ± SD or median (IQR).
*Defined as change from rest to end of exercise.
HR: heart rate; IGR: inert gas rebreathing; PBF: pulmonary blood flow; SV: stroke volume.

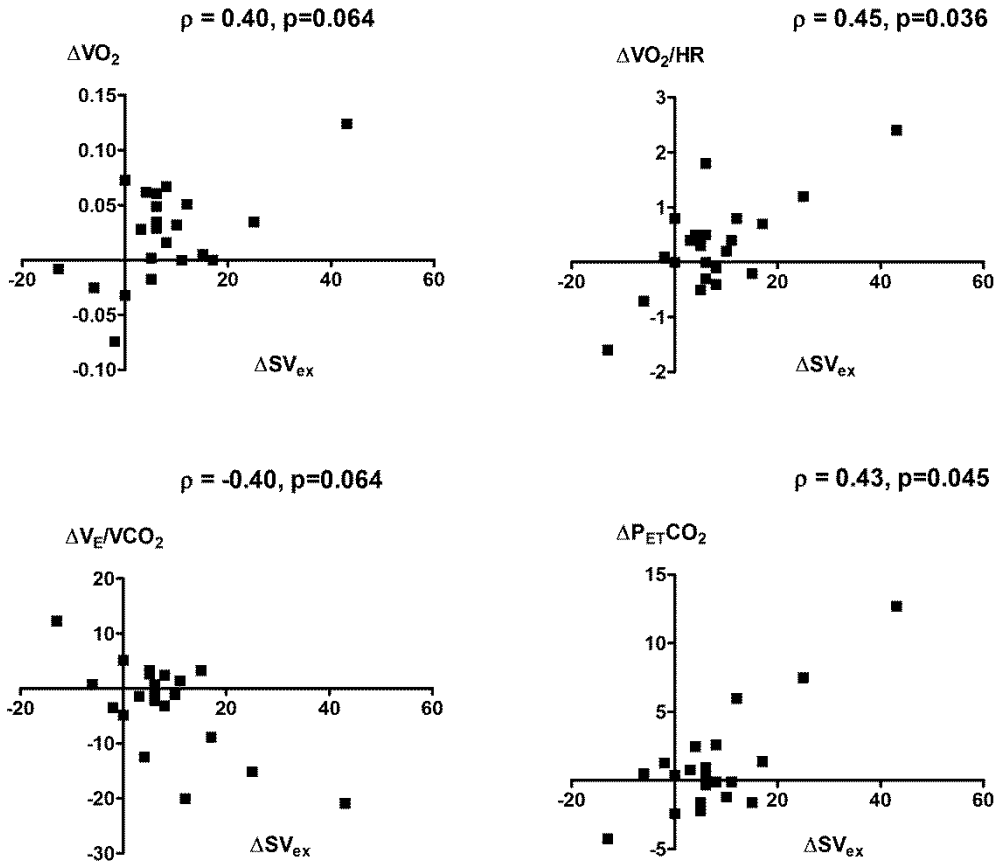
Table 3.4.4. Isotime metabolic measurements during submaximal constant-load exercise

End-exercise values	Baseline	Follow-up	p value
HR, beats/min	113±19	109±20	0.134
VO_2, l/min	0.792±0.197	0.817±0.203	0.010
VO_2/kg, ml/min/kg	10±2	11±2	0.012
VO_2/HR, ml	7±2	8±2	0.079
$C_{a-v}O_2$, ml/dl	16.3±3.8	14.7±3.6	0.021
V_E, l/min	42.1±9.2	39.7±8.4	0.087
V_E/VCO_2	56.3±15.2	54.0±15.5	0.182
V_E/VCO_2 slope	55.6±16.5	52.3±16.7	0.076
$P_{ET}CO_2$, mmHg	23±6	24±6	0.188
S_aO_2, %	92 (88-96)	91±6	0.338
RER	0.90±0.07	0.88±0.06	0.025

Data are expressed as mean ± SD or median (IQR).

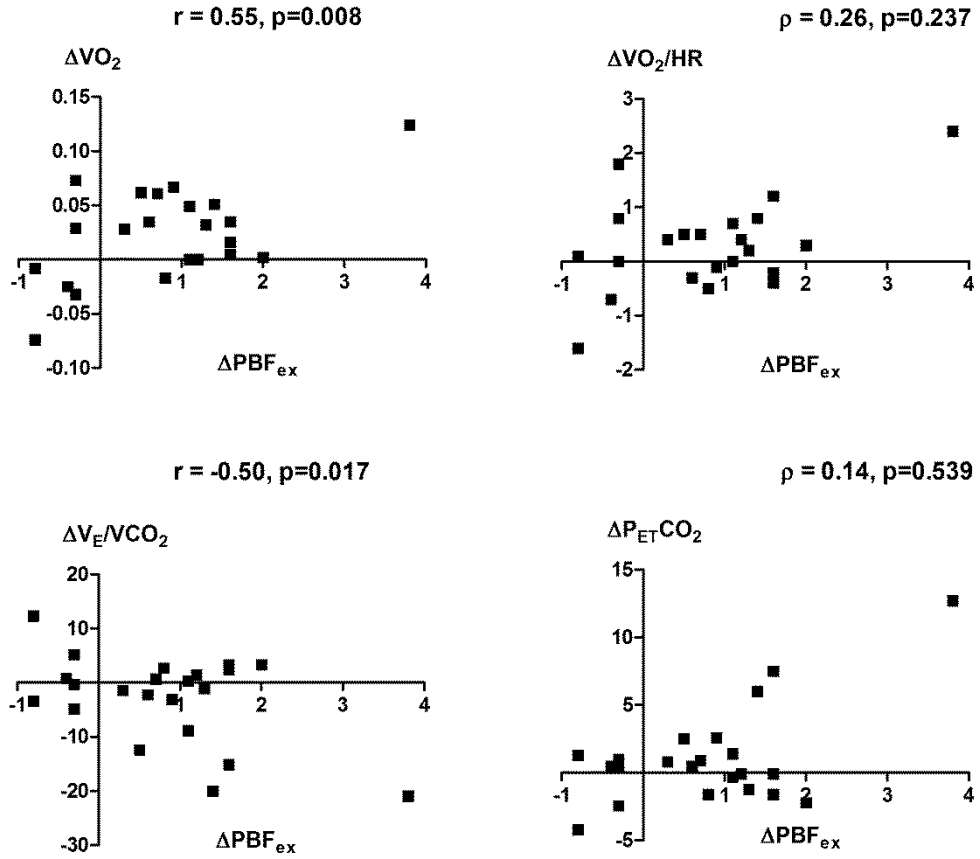
$C_{a-v}O_2$: arteriovenous oxygen content difference; HR: heart rate; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; RER: respiratory exchange ratio; V_E: minute ventilation; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide; S_aO_2: arterial oxygen saturation.

Figure 3.4.1. Correlation between therapy-induced changes in submaximal exercise SV and isotime metabolic measurements



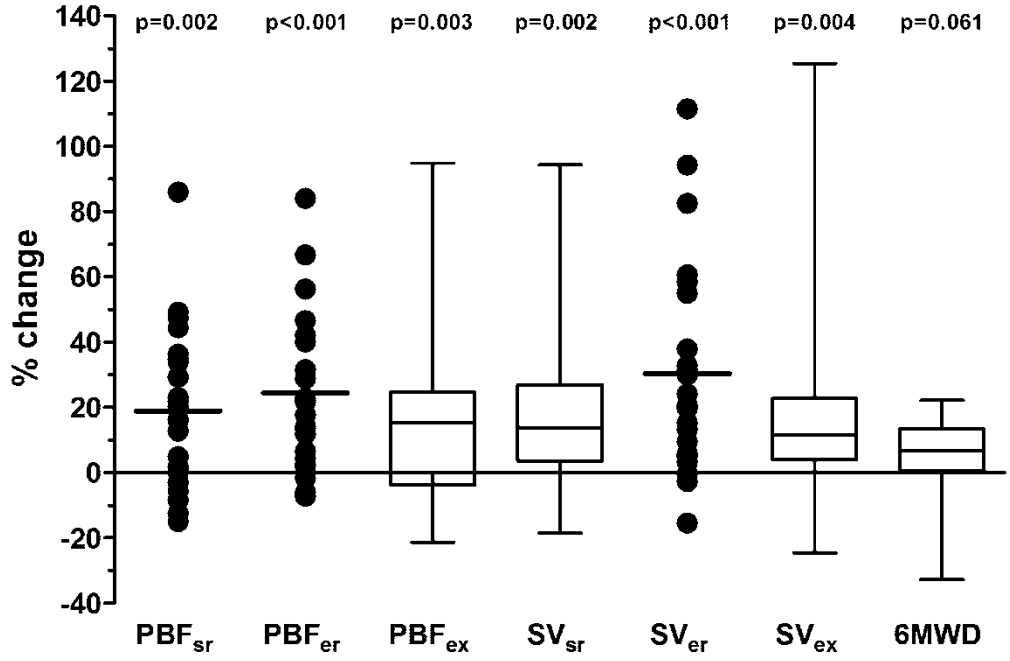
ρ: Spearman correlation coefficient.
$\Delta P_{ET}CO_2$: change in end-tidal carbon dioxide partial pressure; ΔSV_{ex}: change in exercise stroke volume; $\Delta V_E/VCO_2$: change in ventilatory equivalent for carbon dioxide; ΔVO_2: change in oxygen uptake; ΔVO_2/HR: change in oxygen pulse.

Figure 3.4.2. Correlation between therapy-induced changes in submaximal exercise PBF and isotime metabolic measurements



r: Pearson correlation coefficient; ρ: Spearman correlation coefficient.
ΔPBF_{ex}: change in exercise pulmonary blood flow; $\Delta P_{ET}CO_2$: change in end-tidal carbon dioxide partial pressure; $\Delta V_E/VCO_2$: change in ventilatory equivalent for carbon dioxide; ΔVO_2: change in oxygen uptake; ΔVO_2/HR: change in oxygen pulse.

Figure 3.4.3. Percentage changes in IGR measurements and 6MWD from baseline



Non-normally distributed variables are represented by box and whiskers plots. The whiskers represent the minimum and maximum values and the box represents the 25th and 75th percentiles. The line in the box represents the median. Normally distributed variables are represented by dot plots. The line represents the mean. 6MWD: six-minute walk distance; er: erect rest; ex: exercise; IGR: inert gas rebreathing; PBF: pulmonary blood flow; sr: supine rest; SV: stroke volume. p values refer to comparison of variables between baseline and follow-up by paired t test or Wilcoxon signed rank test.

Table 3.4.5 Patient characteristics of 6MWD subgroups

	High 6MWD	Low 6MWD	p value
Patient number, n	12	12	-
Age, years	52±13	70 (59-75)	0.026
Female, n (%)	2 (17)	6 (50)	0.083
Haemodynamics			
RAP, mmHg	9±5	6±4	0.229
mPAP, mmHg	48±15	48±12	0.988
CO, l/min	4.5±1.8	4.1±1.2	0.475
CI, l/min/m^2	2.3±0.8	2.2±0.7	0.641
PVR, Wood units	10.1±5.4	9.8 (6.5-14.9)	0.583
S_vO_2, %	66±10	64±12	0.727
Conventional outcome measures			
6MWD, m	477±80	291±47	<0.001
WHO FC, II/III, n	8/4	4/8	0.103
NT-proBNP, pg/ml	782±816	1442±1599	0.277
CAMPHOR	28±22	38±20	0.274
CPET characteristics			
Peak VO_2, l/min	1.152±0.386	0.789±0.208	0.012
Peak VO_2/HR, ml	8±2	6±2	0.009
V_E/VCO_2 at AT	49.3±8.9	63.1±19.6	0.047
$P_{ET}CO_2$ at AT, mmHg	26±4	22±6	0.055

Data are expressed as mean ± SD, median (IQR) or n (%).
6MWD: six-minute walk distance; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; CI: cardiac index; CO: cardiac output; CPET: cardiopulmonary exercise test; mPAP: mean pulmonary artery pressure; NT-proBNP: N-terminal pro-brain natriuretic peptide; $P_{ET}CO_2$ at AT: end-tidal carbon dioxide partial pressure at anaerobic threshold; PVR: pulmonary vascular resistance; RAP: right atrial pressure; S_vO_2: mixed venous saturation; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2 at AT: ventilatory equivalent for carbon dioxide at anaerobic threshold; WHO FC: World Health Organisation functional class.

Table 3.4.6. IGR measurements during submaximal constant-load exercise in 6MWD subgroups

	High 6MWD group (n=12)			Low 6MWD group (n=12)		
	Baseline	**Follow-up**	**p value**	**Baseline**	**Follow-up**	**p value**
6MWD, m	477±80	477±97	0.991	291±47	325±61	<0.001
PBF, l/min						
Supine rest	4.1±0.9	5.0±1.4	0.013	3.8±1.2	4.3±1.3	0.068
Erect rest	3.3±0.5	4.1±1.0	0.012	3.1±0.7	3.8±0.8	0.004
Exercise	5.6±1.8	6.5±2.1	0.031	5.0±1.7	5.6±1.9	0.030
SV, ml						
Supine rest	55 (38-73)	64±18	0.084	47 (29-62)	57 (37-62)	0.010
Erect rest	43±10	52±14	0.027	37 (24-42)	48±16	0.001
Exercise	52±17	62±21	0.030	44±19	49±19	0.064

Data are expressed as mean ± SD or median (IQR).
6MWD: six-minute walk distance; IGR: inert gas rebreathing; PBF: pulmonary blood flow; SV: stroke volume.

Table 3.4.7. Isotime metabolic measurements during submaximal constant-load exercise in 6MWD subgroups

	High 6MWD group (n=12)			Low 6MWD group (n=12)		
	Baseline	**Follow-up**	**p value**	**Baseline**	**Follow-up**	**p value**
HR, beats/min	108±17	103±16	0.079	118±19	114±22	0.394
VO_2, l/min	0.873±0.220	0.889±0.230	0.332	0.703 (0.605-0.798)	0.731 (0.634-0.817)	0.009
VO_2/kg, ml/min/kg	11±2	12±2	0.297	9 (8-10)	9 (8-11)	0.009
VO_2/HR, ml	8±2	9±3	0.163	6 (5-7)	7 (6-8)	0.203
$C_{a-v}O_2$, ml/dl	16.7±2.5	14.8±3.1	0.054	15.9±4.9	14.7±4.2	0.186
V_E, l/min	42.6±11.1	35.5 (32.4-43.2)	0.045	41.7±7.5	41.5±7.5	0.908
V_E/VCO_2	51.3±13.1	48.6±14.2	0.284	60.9±16.0	58.9±15.5	0.443
V_E/VCO_2 slope	49.9±13.2	43.1 (34.8-58.1)	0.638	61.3±18.0	56.5±16.5	0.157
$P_{ET}CO_2$, mmHg	25±6	26±6	0.229	22±6	23±5	0.632
SaO_2, %	94±3	94±4	1.000	89±6	88±6	0.218
RER	0.91±0.07	0.86±0.06	0.005	0.90±0.06	0.90±0.04	0.942

Data are expressed as mean ± SD or median (IQR).
6MWD: six-minute walk distance; $C_{a-v}O_2$: arteriovenous oxygen content difference; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; RER: respiratory exchange ratio; S_aO_2: arterial oxygen saturation; V_E: minute ventilation; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide.

Correlation between 6MWD and IGR measurements

At baseline, 6MWD correlated with submaximal exercise PBF (r=0.52, p=0.008) and SV (r=0.47, p=0.021). At follow-up, 6MWD correlated with supine rest PBF (r=0.46, p=0.025) and SV (ρ=0.42, p=0.041), submaximal exercise PBF (ρ=0.53, p=0.010) and SV (r=0.54, p=0.008). There was no significant correlation between 6MWD and erect rest IGR measurements or between therapy-induced changes in 6MWD and IGR measurements.

Metabolic measurements during the 6MWT

These results are separately presented and discussed in Chapter 4.

Post-hoc power calculation

This was performed using data on treatment effect and SD observed in this study for conventional outcome measures, IGR and isotime metabolic measurements (table 3.4.8). Much larger sample sizes would be required for conventional outcome measures (50 to 268) and isotime metabolic variables (24 to 100) to detect a treatment effect compared to IGR measurements (13 to 22).

Table 3.4.8. Post-hoc power calculation

	Treatment effect	Required sample size*
Conventional outcome measures		
6MWD, m	17±42	50
WHO FC	0.5 to 0.38†	268
NT-proBNP, pg/ml	131±738	252
CAMPHOR	3±15	199
IGR measurements		
PBF, l/min		
supine rest	0.7±0.9	16
erect rest	0.7±0.8	13
exercise	0.8±1.1	17
SV, ml		
supine rest	7±10	19
erect rest	10±11	12
exercise	7±11	22
Isotime metabolic measurements		
VO_2, l/min	0.025±0.042	24
$C_{a-v}O_2$, ml/dl	1.6±3.0	30
VO_2/HR, ml	0.5±1.3	54
V_E/VCO_2	2.3±8.1	100
$P_{ET}CO_2$, mmHg	1±4	100

* To detect a treatment effect with 80% power and a two-tailed significance level of 0.05 by paired t test.
† To detect a change in the proportion of WHO FC III patients from 0.5 to 0.38 by Chi-squared test.
6MWD: six-minute walk distance; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; $C_{a-v}O_2$: arteriovenous oxygen content difference; IGR: inert gas rebreathing; NT-proBNP: N-terminal pro-brain natriuretic peptide; PBF: pulmonary blood flow; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; SV: stroke volume; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide; WHO FC: World Health Organisation functional class.

3.4.5 Discussion

This study is the first to show that PBF and SV measured non-invasively at rest and during submaximal constant-load exercise by the IGR method could be used to detect the effect of proven PAH-specific therapy in a group of patients with precapillary PH. The sample size required for IGR measurements to detect a treatment effect was considerably smaller than that required for conventional outcome variables, suggesting that IGR measurements may be more sensitive outcome measures. In the post-hoc analysis, IGR measurements were able to demonstrate a treatment effect in both high and low 6MWD groups whereas 6MWD did not change with therapy in the high 6MWD group. This finding would suggest that IGR measurements may be more sensitive than 6MWD in detecting therapy-induced changes in fitter patients.

Improvement in IGR measurements with therapy was observed across supine rest, erect rest and submaximal exercise. In addition, the exercise response in PBF or SV was unchanged with therapy. Therefore, resting IGR measurements appear to be as sensitive as submaximal exercise measurements in detecting a treatment effect in this study population. The prognostic significance of SV has been demonstrated in a previous study using cardiac MRI [44]. In a group of patients with IPAH, a low SV at baseline predicted survival independently. More importantly, change in SV with PAH-specific therapy was more predictive of survival than change in 6MWD in multivariate analysis, confirming a correlation between SV and clinical outcome. The inability of 6MWD to demonstrate a treatment effect in patients with higher 6MWD in this study concurs with the finding from a previous study which showed that haemodynamic indices measured at RHC were more sensitive than 6MWD as outcome measures in patients walking >450 m [68]. The precise mechanism behind this observation is unclear but is thought to be related to the ceiling effect of 6MWD.

Metabolic variables during incremental CPET have been shown to have prognostic significance, but previous studies using these variables as outcome measures in PAH showed mixed results [171]. The use of metabolic variables to

assess treatment response in PH is revisited in this study using isotime comparison at submaximal constant–load exercise. At follow-up there was a small significant increase in VO_2 and decrease in $C_{a-v}O_2$ consistent with an improved exercise CO secondary to therapy, but no change in the metabolic indices of ventilatory efficiency. There is therefore no conclusive evidence from this study to support the use of isotime metabolic variables to assess treatment response, but it does add to the current body of literature on the use of CPET in PAH, an area where more research is required.

Stroke volume measured at supine rest was greater than that at erect and comparable to that at submaximal exercise, as a result of a greater preload and lower PVR in the supine position [172]. This gives rise to a smaller % therapy-induced change in PBF and SV at supine rest compared to erect rest despite similar absolute changes. There was no significant increase in the exercise response of PBF or SV after 3 months of new therapy. This indicates that the increase in submaximal exercise PBF or SV at follow-up was a result of an increase in resting values combined with an unchanged exercise response. The increase in SV with new therapy was not accompanied by an increase in isotime VO_2/HR. This may be owing to the fact that a reduction in SV would be compensated by an increase in $C_{a-v}O_2$ and vice versa, thereby attenuating changes in VO_2/HR [41]. Directly measured SV would therefore be more sensitive than VO_2/HR in detecting clinical change.

There was no correlation between therapy-induced changes in submaximal exercise IGR measurements and 6MWD, and this may be related to the limitations of 6MWD previously discussed. In addition, published reference equations for 6MWD show that only 44-60% of normal inter-subject variance can be explained by physiological factors such as age, gender, height and weight [63-66]. This suggests that there are other undefined determinants of performance at the 6MWT which may outweigh the influence of disease in an individual, especially in those who are mildly affected. These factors would add to the noise of 6MWD measurements and mask any clinical change secondary to therapy. On the other hand, submaximal exercise IGR measurements are

markers of PH-specific change and unaffected by patient volition or a ceiling effect.

Although the findings in this study are preliminary, the use of IGR measurements in PH warrants further studies, especially in specific patient groups. IGR measurements would allow more reliable assessment of therapy-induced changes in patients with comorbid conditions affecting 6MWD, such as patients with connective tissue disease. They may be superior to 6MWD as outcome measures in clinical trials focused on patients with less advanced disease. Resting measurements could be used to assess patients who are too disabled to perform a 6MWT. In addition, simultaneous measurement of SV and VO_2 would allow determination of $C_{a-v}O_2$, which may be reduced in patients with peripheral muscle dysfunction confounding exercise limitation, a recognised phenomenon in PAH [164]. In this setting, exercise training may have an adjunctive role to medical therapy in improving exercise capacity [165;166].

This study has a number of limitations. It was underpowered to detect changes in WHO FC, NT-proBNP and CAMPHOR score with new therapy. The aetiology of PH in the study population was heterogeneous, including patients with CTEPH and one patient with sarcoidosis, but this reflects the real-life nature of the study population. Although PAH-specific therapy is currently not approved in CTEPH, there is some evidence from several uncontrolled studies and one randomised controlled trial to support its efficacy. Its off-label use in patients with inoperable disease, persistent PH after surgery or as a bridge to surgical intervention is recommended by current guidelines [2;5;173]. In sarcoidosis, the mechanisms limiting exercise may differ from PAH or CTEPH, but excluding this patient did not affect the results of the analyses. Patients in WHO FC IV were not included as they were unable to perform exercise tests. Prevalent patients were included whose response to new therapy might be less marked than that seen in treatment-naive patients, but the number was small and this would not have affected the comparison between 6MWD and IGR measurements. Repeat RHC data were not available at follow-up to assess treatment efficacy, but this was not the aim of the study. The present study was designed to determine if

the effect of proven PAH-specific therapy could be detected by IGR measurements alongside conventional outcome measures in patients with precapillary PH representative of those seen in routine clinical practice. Lastly, in the absence of a placebo-controlled arm, we cannot completely exclude the possibility that changes in IGR measurements after new or modified PAH treatment had occurred by chance, which is an inherent problem with real-life observational data. To confirm the results from this study, the ability of IGR measurements to detect treatment effect should ideally be compared with 6MWD and other conventional outcome measures in randomised controlled drug trials.

3.4.6 Conclusions

This study is the first to show that non-invasive resting and submaximal exercise IGR haemodynamic measurements could be used to detect treatment response in precapillary PH. The data analysis also suggests that these measurements may be more sensitive than 6MWD in detecting the effects of therapy in fitter subjects. In comparison, other conventional outcome measures and isotime metabolic variables were less responsive to change.

4 USE OF END-TIDAL CARBON DIOXIDE PARTIAL PRESSURE TO DETECT TREATMENT RESPONSE IN PRECAPILLARY PULMONARY HYPERTENSION

4.1 Summary

Rationale: $P_{ET}CO_2$ is a metabolic index of ventilatory efficiency and is reduced at rest and during exercise in patients with PH in proportion to disease severity. The aim of this study was to determine if $P_{ET}CO_2$ measured during the 6MWT could be used to detect treatment response in patients with precapillary PH.

Methods: Twenty four patients with precapillary PH receiving de novo or modified PAH-specific therapy were studied. Distance walked and metabolic variables during the 6MWT were measured at baseline and after 3 months of new therapy.

Results: Metabolic data during the 6MWT were available for analysis in 20 patients. VO_2, VCO_2, V_E, HR, VO_2/HR and V_E/VCO_2 increased while $P_{ET}CO_2$ decreased towards a plateau during the test. RER increased and S_aO_2 decreased progressively throughout the test. Compared with incremental CPET, patients had greater arterial desaturation and reached a lower peak V_E during the 6MWT. At follow-up, there were trended improvements in 6MWD (384±115 to 401±111 m, p=0.061) and end-of-walk RER (1.09±0.16 to 1.03±0.14, p=0.068), but no significant changes in other metabolic variables. Therapy-induced changes in 6MWD correlated with changes in end-of-walk VO_2 (ρ=0.67, p=0.003), VO_2/HR (ρ=0.50, p=0.004), V_E/CO_2 (ρ=0.46, p=0.049) and the nadir of $P_{ET}CO_2$ (ρ=0.48, p=0.039).

Conclusions: The study provided further insights into the ventilatory response during the 6MWT, but did not yield conclusive information on the use of $P_{ET}CO_2$ during the 6MWT to detect treatment response in patients with precapillary PH due to its small sample size.

4.2 Introduction

Six-minute walk distance is used as a simple surrogate of peak exercise capacity in PH, but observed increase in 6MWD with therapy may not reflect improved right heart function or haemodynamic status, and could be brought about by other unrelated mechanisms such as improvements in walking efficiency or subject motivation. Measuring the ventilatory and gas exchange responses during 6MWT would provide mechanistic insights into improved performance and allow assessment of PH-specific changes. This was performed in a group of 20 PAH patients, and the results were compared with those obtained during a standard incremental CPET [69]. Deboeck et al showed that VO_2, VCO_2, V_E, VO_2/HR and HR increased whereas S_aO_2 decreased in the first 2 to 3 min of the 6MWT and reached a plateau thereafter. V_E/VCO_2 increased in the first min, then returned to baseline value and remained constant. The walking speed was on average constant throughout the 6MWT. Compared with incremental CPET, VO_2 achieved during 6MWT tended to be higher than peak VO_2, whereas V_E, RER and HR were significantly lower. Plateau VO_2/HR and arterial desaturation were greater during the 6MWT. Plateau V_E/VCO_2 during the 6MWT was equivalent to that observed at AT of the CPET. These findings support the notion that the 6MWT corresponds to a sustainable submaximal aerobic effort, and that patients with PAH exercise at a higher aerobic capacity but lower ventilatory stress during the 6MWT compared with a standard incremental CPET. A recent study showed that resting $P_{ET}CO_2$ measured at the beside using a handheld capnograph could be used to differentiate patients with PAH from those with pulmonary venous hypertension and those without PH, and that resting $P_{ET}CO_2$ improved in patients who had clinical response to new or escalated epoprostenol therapy [174]. However, no studies have looked at changes in ventilatory variables during the 6MWT and the mechanisms behind improved 6MWD following PAH-specific therapy.

A low $P_{ET}CO_2$ at rest, AT and peak exercise is characteristic of the CPET response in PH patients. The degree of reduction correlates with disease severity [175] and has prognostic significance [94]. $P_{ET}CO_2$ at rest and during exercise normalise with

effective therapy and therefore may be a potential marker of treatment response. $P_{ET}CO_2$ is reproducible and can be readily measured using a handheld capnograph as demonstrated in a study on the use of bedside $P_{ET}CO_2$ to detect pulmonary embolism [176]. The aim of this study was to determine if $P_{ET}CO_2$ during the 6MWT could be used to detect treatment response in patients with precapillary PH.

4.3 Methods

4.3.1 Study subjects

This study was carried out in conjunction with the study described in Chapter 3.4. The inclusion and exclusion criteria are as described in Chapter 3.4.3.

4.3.2 Study design

This is described in Chapter 3.4.3. In brief, study patients underwent a symptom-limited incremental CPET and a 6MWT at baseline. On a separate day within 2 weeks, they performed a second 6MWT with ventilatory measurements. This was repeated after 3 months of new PAH-specific therapy.

4.3.3 Ventilatory measurements during 6MWT

The use of a portable metabolic device during 6MWT is described in Chapter 2.5.2. VO_2, VCO_2, V_E, V_E/VO_2, V_E/VCO_2, VO_2/HR, $P_{ET}O_2$, $P_{ET}CO_2$, RER, HR and S_aO_2 were measured continuously for 3 min at rest, during the walk test and for 3 min in recovery. The values achieved at the end of the 6MWT were used for comparison between baseline and follow-up 6MWT, and with the peak values achieved at the incremental CPET. Exercise response is defined as changes in ventilatory variables from rest to the end of the 6MWT. The physiological response during the 6MWT was studied by plotting VO_2, HR, VO_2/HR, V_E, V_E/VCO_2, $P_{ET}CO_2$ and S_aO_2 against time.

4.3.4 Statistical analysis

Statistical analysis was performed using Statview version 5.0.1 (SAS Institute, Cary, NC, USA) and Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Continuous variables were tested for normality using D'Agostino and Pearson omnibus normality test. Normally distributed variables are shown as mean ± SD and non-normally distributed variables are shown as median (IQR). Categorical variables are presented as number (%). Comparison of ventilatory variables between baseline 6MWT and incremental CPET, and that between baseline and follow-up 6MWT were made by paired t test or Wilcoxon signed rank test depending on data distribution. Post-hoc analysis was carried out by stratifying patients into high and low 6MWD groups using the median baseline 6MWD. Correlation between two variables that were both normally distributed was determined by Pearson correlation coefficient (r), and otherwise by Spearman correlation coefficient (ρ). A p value<0.05 was defined as statistically significant.

4.4 Results

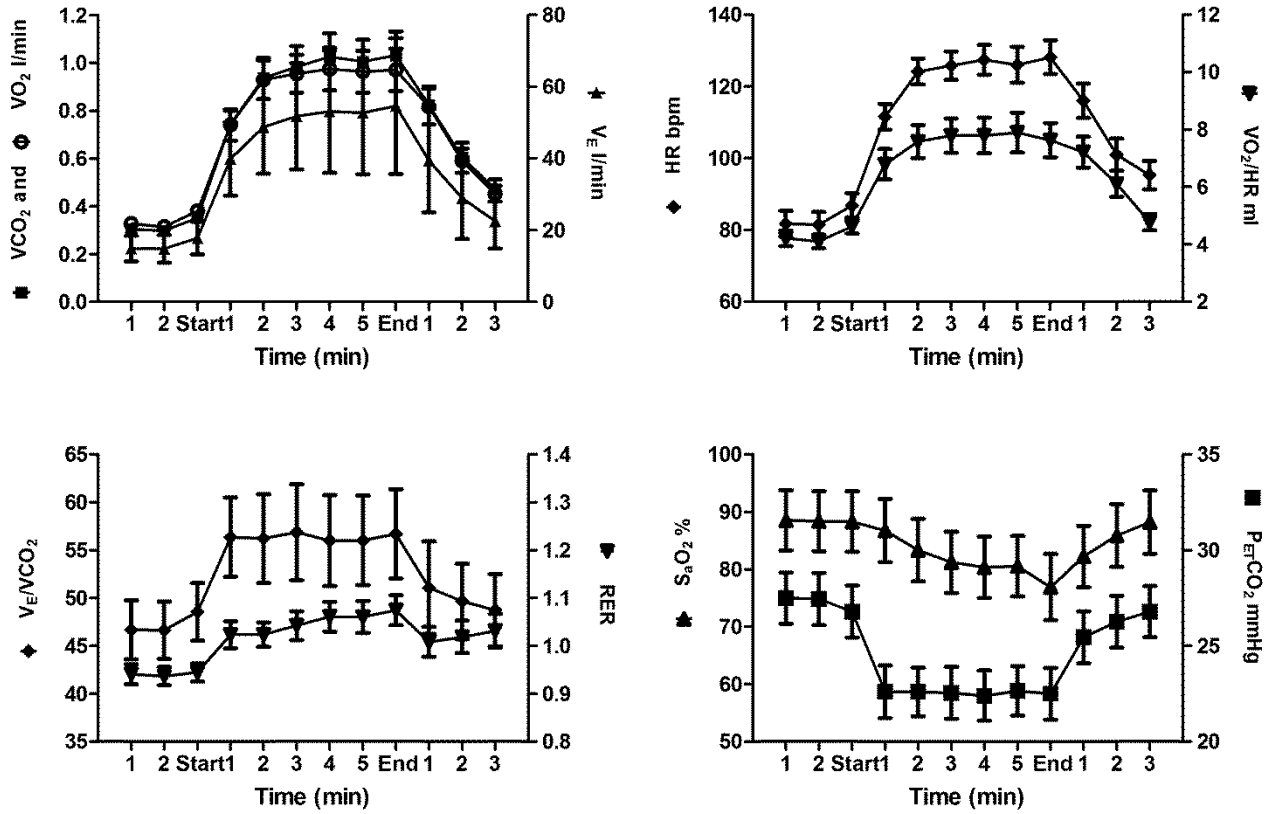
4.4.1 Patient characteristics

The results are described in Chapter 3.4.4

4.4.2 Ventilatory response during the 6MWT

The ventilatory and gas exchange responses during the 6MWT are shown in figure 4.1. VO_2, VCO_2, V_E, HR and VO_2/HR increased rapidly in the first 2 min, then at a slower rate reaching a plateau at 4 min. $P_{ET}CO_2$ decreased and V_E/VCO_2 increased in the first min and reached a plateau thereafter. RER increased and S_aO_2 decreased progressively throughout the test.

Figure 4.1. Changes in oxygen uptake, carbon dioxide output, heart rate, oxygen pulse, ventilatory equivalent for carbon dioxide, respiratory exchange ratio, oxygen saturation and end-tidal carbon dioxide partial pressure during the 6MWT



6MWT: 6-minute walk test; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; RER: respiratory exchange ratio; S_aO_2: arterial oxygen saturation; V_E: minute ventilation; VCO_2: carbon dioxide output; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide.

4.4.3 Comparison between 6MWT and CPET

Baseline 6MWD correlated with peak VO_2 measured at incremental CPET (r=0.74, p<0.001). Patients achieved a lower V_E but experienced a greater degree of arterial desaturation during the 6MWT compared with incremental CPET (table 4.1). No significant differences were demonstrated in VO_2, VO_2/HR, HR and RER. V_E/VCO_2 and $P_{ET}CO_2$ achieved at the end of the 6MWT were comparable to those obtained at the AT of incremental CPET.

Table 4.1. Comparison between 6MWT and incremental CPET

	6MWT (end-of-walk values)	CPET (peak values)	p value
HR, beats/min	126±20	129±27	0.790
VO_2, l/min	0.927±0.271	0.865 (0.673-0.953)	0.623
VO_2/kg, ml/min/kg	12±3	12±3	0.283
VO_2/HR, ml	7±2	7±2	0.049
V_E, l/min	53.1±14.6	64.2±16.9	<0.001
V_E/VCO_2	54.2±14.7	52.0 (48.0-66.5)*	0.205
$P_{ET}CO_2$, mmHg	22±6	24±6*	0.112
S_aO_2, %	86±7	91±6	<0.001
RER	1.09±0.16	1.15±0.16	0.253

Data are expressed as mean ± SD or median (IQR).
*measured at anaerobic threshold.
6MWT: 6-min walk test; CPET: cardiopulmonary exercise test; HR: heart rate; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; S_aO_2: arterial oxygen saturation; RER: respiratory exchange ratio; V_E: minute ventilation; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide.

4.4.4 Changes in ventilatory variables with therapy

After 3 months of new therapy, 6MWD improved from 384±115 to 401±111 m (mean change 17±42 m; median change 29 (3-47) m, p=0.061). There were no significant changes in resting, end-of-walk $P_{ET}CO_2$ or $P_{ET}CO_{2\ nadir}$. Apart from a trended decrease in end-of-walk RER, there were no significant changes in other ventilatory variables (table 4.2). In the post-hoc analysis, 6MWD improved by 34±25 m (<0.001) in the low 6MWD group (291±47 m at baseline) whereas it did not change significantly in the high 6MWD group (477±80 m at baseline). In contrast, none of the ventilatory variables changed significantly in the low 6MWD group, but significant improvements were seen in end-of-walk HR (127±16 versus 120±15 beats/min, p=0.002) and in the exercise response of VO_2/HR (4±2 versus 5±2 ml, p=0.037), HR (50±14 versus 45±9 beats/min, p=0.013) and $P_{ET}CO_2$ (-7±5 versus -3±3 mmHg , p=0.028) in the high 6MWD group. Baseline characteristics of the 6MWD subgroups are shown table 3.4.5

4.4.5 Correlations of therapy-induced changes

Therapy-induced changes in 6MWD correlated with those in end-of-walk VO_2 (ρ=0.67, p=0.003), VO_2/HR (ρ=0.50, p=0.004) and V_E/VCO_2 (ρ=-0.46, p=0.049), and $P_{ET}CO_{2\ nadir}$ (ρ=0.48, p=0.039) (figure 4.2). There were no significant correlations between therapy-induced changes in 6MWD and those in end-of-walk $P_{ET}CO_2$, V_E, HR, RER or S_aO_2.

4.4.6 Correlations between $P_{ET}CO_2$ and conventional outcome measures

Resting $P_{ET}CO_2$ correlated with 6MWD at baseline (r=0.57, p=0.014) and at follow-up (r=0.56, p=0.017) and WHO FC at baseline (ρ=-0.53, p=0.025). End-of-walk $P_{ET}CO_2$ correlated with 6MWD at baseline (r=0.53, p=0.021) and at follow-up (r=0.73, p<0.001), WHO FC at baseline (ρ=-0.52, p=0.023), NT-proBNP at baseline (r=-0.61, p=0.016) and at follow-up (r=-0.56, p=0.029). $P_{ET}CO_{2\ nadir}$ correlated with 6MWD at baseline (r=0.59, p=0.007) and at follow-up (r=0.74, p<0.001), WHO FC at baseline (ρ=-0.50, p=0.029), NT-proBNP at baseline (r=-

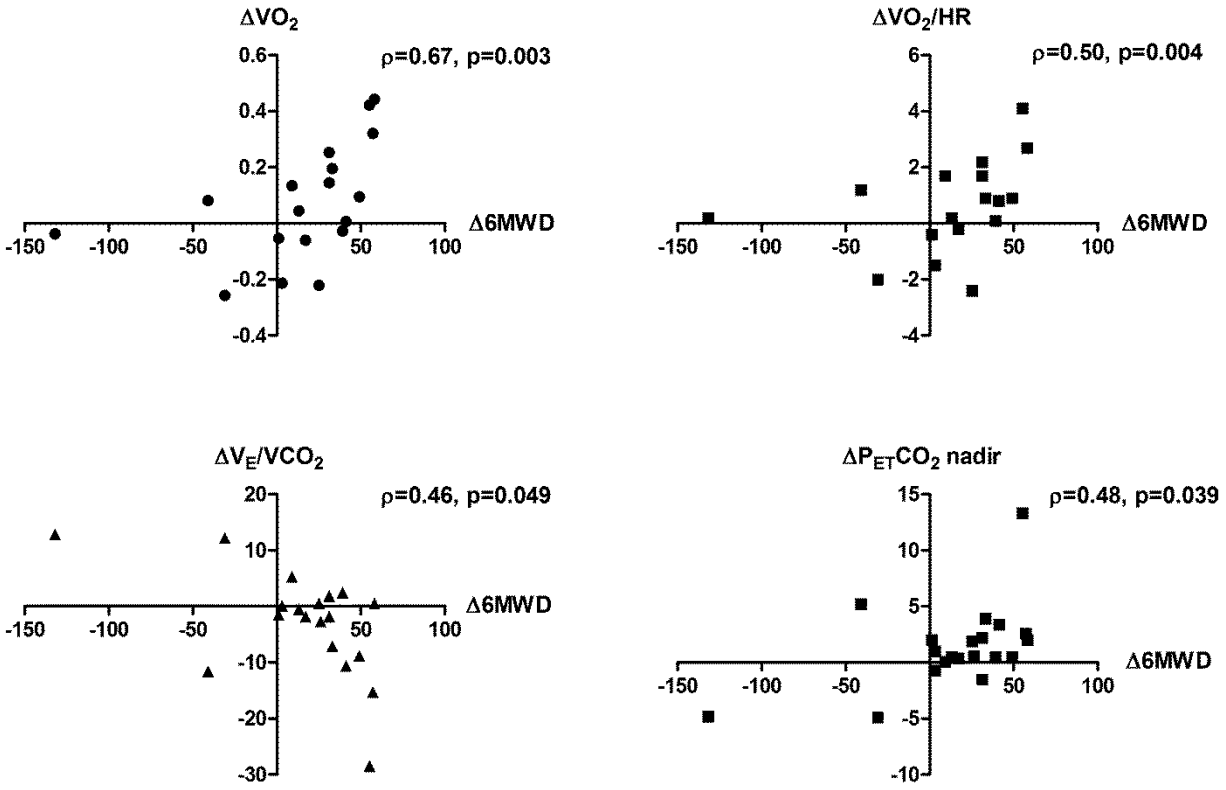
0.54, p=0.050) and at follow-up (r=-0.55, p=0.036), and CAMPHOR score at follow-up (r=-0.50, p=0.043).

Table 4.2. Ventilatory measurements during the 6MWT

End-of-walk	Baseline	Follow-up	p value
HR, beats/min	126±20	124±11	0.498
VO_2, l/min	0.927±0.271	0.998±0.304	0.162
VO_2/kg, ml/min/kg	12±3	13±3	0.302
VO_2/HR, ml	7±2	8±3	0.161
V_E, l/min	53.1±14.6	51.4±14.5	0.452
V_E/VCO_2	54.2±14.7	51.4±15.2	0.205
V_E/VCO_2 slope	59.1±17.9	55.1±18.5	0.153
Resting $P_{ET}CO_2$, mmHg	29±7	28±6	0.862
$P_{ET}CO_2$, mmHg	22±6	23±6	0.162
$P_{ET}CO_{2\ nadir}$, mmHg	21±6	22±6	0.108
S_aO_2, %	86±7	86±8	0.720
RER	1.09±0.16	1.03±0.14	0.068

Data are expressed as mean ± SD or median (IQR).
6MWT: six-minute walk test; HR: heart rate; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; $P_{ET}CO_{2\ nadir}$: the lowest point of end-tidal carbon dioxide partial pressure; RER: respiratory exchange ratio; S_aO_2: arterial oxygen saturation; V_E: minute ventilation; VO_2: oxygen uptake; VO_2/HR: oxygen pulse; V_E/VCO_2: ventilatory equivalent for carbon dioxide.

Figure 4.2. Correlation between therapy-induced changes in 6MWD and ventilatory variables



Δ6MWD: change in 6-minute walk distance; $\Delta P_{ET}CO_2$ nadir: change in nadir end-tidal carbon dioxide partial pressure; ΔVO_2: change in oxygen uptake; ΔVO_2/HR: change in oxygen pulse; $\Delta V_E/VCO_2$: change in ventilatory equivalent for carbon dioxide.

4.5 Discussion

In this study, the effect of new PAH-specific therapy on the ventilatory response during the 6MWT was examined in a group of patients with precapillary PH. A trended improvement in 6MWD with therapy change was not accompanied by significant changes in ventilatory variables. However the observed moderate correlations between therapy-induced changes in 6MWD and those in VO_2, VO_2/HR, V_E/VCO_2 and $P_{ET}CO_{2\ nadir}$ would fit in with the mechanisms behind improved 6MWD. In addition, PH patients were found to exercise at a higher aerobic capacity with a greater degree of arterial desaturation but at lower ventilatory stress at the 6MWT compared with incremental CPET, confirming the findings of a previous study.

Assuming the influences of subject volition and learning effect are negligible, improved 6MWD with new PAH-specific therapy would reflect improvements in exercise CO response and/or ventilatory efficiency. Accordingly, concurrent improvements in the CPET indices of cardiac function (VO_2, VO_2/HR) and/or ventilatory efficiency (V_E/VCO_2, $P_{ET}CO_2$) would be expected. However, no significant changes in these variables were detected during the 6MWT at follow-up. In the post-hoc analysis, 6MWD was found to be less sensitive in detecting treatment response in the high 6MWD group compared with the low 6MWD group (discussed in Chapter 3.4). When analyses of the ventilatory response during the 6MWT were stratified by 6MWD subgroups, no changes in ventilatory variables with therapy were demonstrated in the low 6MWD group despite an improved 6MWD, whereas several variables improved significantly in the absence of improved 6MWD in the high 6MWD group. This could be the result of statistical irregularities related to the small sample size, but does raise the possibility that ventilatory variables may be more sensitive than the distance walked during the 6MWT in detecting a treatment effect in patients walking >450 m.

Based on a SD of 6mmHg observed in this study, the sample size required to detect a treatment effect in $P_{ET}CO_2$ of 2mmHg with 80% power and 5% two-tailed level of significance by paired t test would be 73. On the contrary, a recent study was able to demonstrate an improvement in resting $P_{ET}CO_2$ in only 14 PAH patients receiving new or escalated epoprostenol therapy [174]. The fact that epoprostenol has more potent pulmonary vasodilatory effects than other oral agents may partly explain this discrepancy.

4.6 Conclusions

This study was underpowered to detect a change in $P_{ET}CO_2$ during the 6MWT with new therapy, hence no conclusions can be drawn on its use to detect treatment response in patients with precapillary PH. However, it has confirmed previous findings on the ventilatory response during the 6MWT in PH patients, and more importantly provided useful preliminary data for future studies in this field.

5 THE ROLE OF PERCENT PREDICTED SIX-MINUTE WALK DISTANCE IN PULMONARY ARTERIAL HYPERTENSION

5.1 Summary

Rationale: Absolute 6MWD predicts mortality in PAH, but varies greatly between normal individuals due to physiological factors such as age, gender, height and weight. The % predicted 6MWD adjusts for these factors and may predict mortality more reliably. The aim of the study was to compare the strength of mortality prediction by absolute and % predicted 6MWD in PAH at baseline and on treatment.

Methods: % predicted 6MWD was calculated using four different reference equations in 137 IPAH and CTDPAH patients diagnosed between November 2000 and November 2009. Cox proportional hazards and ROC analyses were used to compare the prognostic strength of absolute and % predicted 6MWD.

Results: % predicted 6MWD was predictive of all-cause mortality at baseline (hazard ratios: 0.74-0.83 per 10% increase, $p<0.05$) and on treatment (0.67-0.75 per 10% increase, $p<0.01$), but each respective area under the ROC curve was not significantly different from that of absolute 6MWD for predicting 2-year mortality at baseline (absolute versus % predicted 6MWD: 0.74 versus 0.71-0.75) or on treatment (0.77 versus 0.72-0.78).

Conclusions: % predicted 6MWD may help clinicians interpret the 6MWT, but its prognostic value is not superior to that of absolute 6MWD.

5.2 Introduction

Absolute 6MWD has been used as the primary end-point in most clinical trials of new PAH-specific therapy [2] and remains the principal outcome measure in PH. As a measure of submaximal exercise capacity, it correlates with variables of maximal CPET such as peak VO_2 and VO_2/HR [46] and ventilatory equivalent measured at exercise treadmill testing [47]. In addition, it correlates with markers of disease severity in PAH, such as WHO FC and pulmonary haemodynamics [46]. Most importantly, the ability of baseline 6MWD to predict mortality has been demonstrated in a 12-week randomised controlled trial of intravenous epoprostenol therapy in IPAH [45], the only clinical trial showing a survival benefit of PAH-specific therapy. This was confirmed by subsequent observational studies on the long-term impact of PAH-specific therapy [57-60]. However, there is ongoing debate as to whether % predicted 6MWD should be used instead of absolute 6MWD as it gives a more accurate reflection of the functional impact of disease on an individual.

As with other measurements of physical function, such as lung function and cardiopulmonary exercise capacity, 6MWD varies greatly between individuals due to physiological factors such as age, gender, height and weight, and pathological factors such as cardiopulmonary and musculoskeletal diseases. Adjusting for physiological variation should help clinicians interpret the result of the 6MWT and estimate the degree of exercise impairment due to disease in an individual. Several reference equations already exist to calculate % predicted 6MWD based on an individual's age, gender, height and weight [63-66]. They have been derived from healthy adults sampled from populations in North America and Europe. What is not known is whether % predicted 6MWD is superior to absolute 6MWD at predicting mortality in PAH. The aim of this study was, therefore, to compare the relative strength of mortality prediction by absolute and % predicted 6MWD in PAH measured at baseline and on treatment, respectively, using four different published reference equations.

5.3 Methods

5.3.1 Study subjects

The study cohort was derived by a retrospective case note review of patients referred to the SPVU between November 2000 and November 2009. All consecutive incident cases of IPAH and CTDPAH treated with PAH-specific therapy were identified. Patients with recorded 6MWD at diagnosis or within 12 months of starting treatment were included in this study. Patients with significant lung disease defined by lung function were excluded.

5.3.2 Study design

Baseline data including age, height, weight, lung function, 6MWD, WHO FC, RAP, mPAP, PVR, CI and S_vO_2, and follow-up data including 6MWD, WHO FC and all-cause mortality were collected. Predicted 6MWD in each patient was calculated based on their age, gender, height and weight using each of four reference equations (table 5.1). Absolute 6MWD was expressed as a percentage of the predicted 6MWD.

5.3.3 Statistical analysis

Statistical analysis was performed using Statview version 5.0.1 (SAS Institute, Cary, NC, USA) and Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Continuous variables were tested for normality using D'Agostino and Pearson omnibus normality test. Normally distributed variables are shown as mean ± SD and non-normally distributed variables are shown as median (IQR). Categorical variables are presented as number (%). Comparison of baseline characteristics between IPAH and CTDPAH patients was made by unpaired Student's t test or Mann-Whitney U test depending on data distribution. Changes in absolute and % predicted 6MWD with PAH-specific therapy were assessed using paired Student's t test. Comparison of WHO FC between IPAH and CTDPAH patients at baseline and changes with PAH-specific therapy were assessed using Chi-squared test. The relationship between 6MWD (absolute and each expression

of % predicted 6MWD) and other clinical markers of disease severity was determined by Pearson correlation coefficient if both variables are normally distributed or otherwise by Spearman correlation coefficient.

Survival time was defined as time from the date of RHC to date of death or data cut-off on 30th November 2009. Patients who received lung transplantation or who were lost to follow-up were censored at the time of procedure or last clinical contact (clinic visit, telephone contact or renewal of drug prescription). Univariate Cox proportional hazards analysis was carried out to determine the association between all-cause mortality and age, gender, aetiology, lung function, haemodynamics, WHO FC, absolute 6MWD and each expression of % predicted 6MWD at baseline, and WHO FC, absolute 6MWD and each expression of % predicted 6MWD on treatment. Multiple bivariate Cox models, one for absolute 6MWD and one for each expression of % predicted 6MWD at baseline, were derived by including variables with $p<0.2$ in the univariate analysis to adjust for the effect of confounding. Correlation between candidate variables and the predictor of primary interest (absolute or % predicted 6MWD) was checked to limit collinearity. The variables would not be considered for inclusion if r or ρ is >0.70. The same variables were included to adjust 6MWD in each model to facilitate comparison. The bivariate Cox analysis was repeated for absolute 6MWD and each expression of % predicted 6MWD on treatment. Date of follow-up 6MWD was used as the index date for determining survival time for the analysis on treatment.

To compare the relative prognostic strength among expressions of 6MWD against each other at baseline and on treatment respectively, and each corresponding expression of 6MWD at baseline versus on treatment, ROC analysis was used to determine their respective AUC for predicting 2-year mortality. The significance of differences in AUC was assessed using a paired z test. 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. A p value <0.05 was defined as statistically significant.

Table 5.1. Published reference equations for predicting 6MWD in healthy adults

	Publication year	Study populations	Reference equations	R^2
Enright [64]	1998	USA; 117 males,173 females; age 40-80 years	males: (7.57 x Ht) – (5.02 x age) – (1.76 x Wt) – 309 females: (2.11 x Ht) – (5.78 x age) – (2.29 x Wt) + 667	0.40
Troosters [66]	1999	Belgium; 29 males, 22 females; age 50-85 years	218 + (5.14 x Ht) – (5.32 x age) – (1.8 x Wt) + (51.31 x gender) male=1, female=0	0.66
Gibbons [65]	2001	Canada; 41 males, 38 females; age 20-80 years	868.8 – (age x 2.99) – (gender x 74.7) male=0, female=1	0.41
Chetta [63]	2006	Italy; 48 males, 54 females; age 20-50 years	518.853 + (1.25 x Ht) – (2.816 x age) – (39.07 x gender) male=0, female=1	0.42

6MWD: six-minute walk distance; Ht: height in cm; Wt: weight in kg. R^2 indicates the degree of inter-subject variance explained by the equation.

5.4 Results

5.4.1 Patient characteristics

137 patients were included in the cohort (IPAH 86, CTDPAH 51). The baseline characteristics of the study patients are outlined in table 5.2. During follow-up (median 2 years; range 1.2 months to 8.8 years), 41 patients died from all causes (IPAH 20, CTDPAH 21), two patients were lost to follow-up and two patients received lung transplantation. After receiving PAH-specific monotherapy (7% prostacyclin analogues, 46% endothelin receptor antagonists and 47% phosphodiesterase-5 inhibitors) for 4.1±1.8 months (median 3.5 months; range 2.2 to 11 months), absolute 6MWD and each expression of % predicted 6MWD were 311±111 m, 59±18% (Enright [64]), 48±15% (Troosters [66]), 48±16% (Gibbons [65]) and 58±19% (Chetta [63]) respectively (n=110, all $p<0.001$ versus baseline). On treatment, 41% of patients were in WHO FC II, 54% in III and 4% in IV (n=123, $p<0.005$ versus baseline). At the time of death or censoring, 69% of patients remained on monotherapy and 31% were on combination therapy.

Table 5.2. Patient characteristics at baseline

	All patients	Aetiology		p value*
		IPAH	CTDPAH	
Patients, n	137	86	51	
Age, years	60 (48-70)	60 (41-70)	62 (56-69)	0.175
Female, n (%)	95 (69)	54 (63)	41 (80)	0.031
Height (cm)	164±9	165±9	161±9	0.003
Weight (kg)	73±21	77±19	68±23	0.019
Lung function, % predicted				
FEV_1	87 (75-97)	88 (76-96)	85 (74-99)	0.844
FVC	99 (86-109)	101 (87-109)	98 (81-113)	0.482
DLco	43 (29-61)	45 (28-66)	41 (32-50)	0.218
WHO FC, n (%)				
I and II	22 (16)	17 (20)	5 (10)	0.026
III	104 (76)	59 (69)	45 (88)	–
IV	11 (8)	10 (12)	1 (2)	–
Haemodynamics				
RAP, mmHg	6 (4-11)	6 (3-10)	7 (4-12)	0.399
mPAP, mmHg	48 (38-57)	50 (42-60)	42 (35-54)	0.088
CI, l/min/m^2	2.1 (1.7-2.6)	2.2 (1.7-2.6)	2.0 (1.7-2.6)	0.924
PVR, Wood units	10.5 (7.1-15.5)	10.6 (7.4-15.4)	9.7 (5.9-15.9)	0.357
S_vO_2, %	63±8	64±8	63±9	0.598
6MWD†				
Absolute 6MWD, m	264±111	271±117	251±102	0.330
6MWD, % predicted				
Enright	50±19	50±20	50±19	0.946
Troosters	41±16	41±16	42±15	0.844
Gibbons	41±16	41±16	40±15	0.808
Chetta	49±19	49±20	49±18	0.921

Data are expressed as mean ± SD, median (IQR) or n (%).

*Comparing IPAH versus CTDPAH patients. †n=130.

6MWD: six-minute walk distance; CI: cardiac index; CTDPAH: connective tissue disease associated pulmonary arterial hypertension; DLco: diffusing capacity for carbon monoxide; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; IPAH: idiopathic pulmonary arterial hypertension; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; RAP: right atrial pressure; SvO_2: mixed venous saturation; WHO FC: World Health Organisation functional class.

5.4.2 Correlation with other markers of disease severity

Baseline absolute and % predicted 6MWD correlated weakly with RAP (absolute 6MWD ρ= -0.23, $p<0.01$ versus % predicted 6MWD by all equations ρ= -0.23 to -0.25, $p<0.01$), CI (ρ= 0.24, $p<0.01$ versus ρ= 0.21 to 0.22, $p<0.05$), S_vO_2 (r= 0.21, $p<0.05$ versus r= 0.19-0.21, $p<0.05$) and WHO FC (ρ= -0.33, $p<0.0005$ versus ρ= -0.32 to -0.34, $p<0.005$). Only % predicted 6MWD by Enright and Troosters equations correlated weakly with PVR (ρ= -0.23 and ρ= -0.19, both $p<0.05$). Neither absolute 6MWD nor % predicted 6MWD correlated with mPAP.

5.4.3 Independent effect on mortality

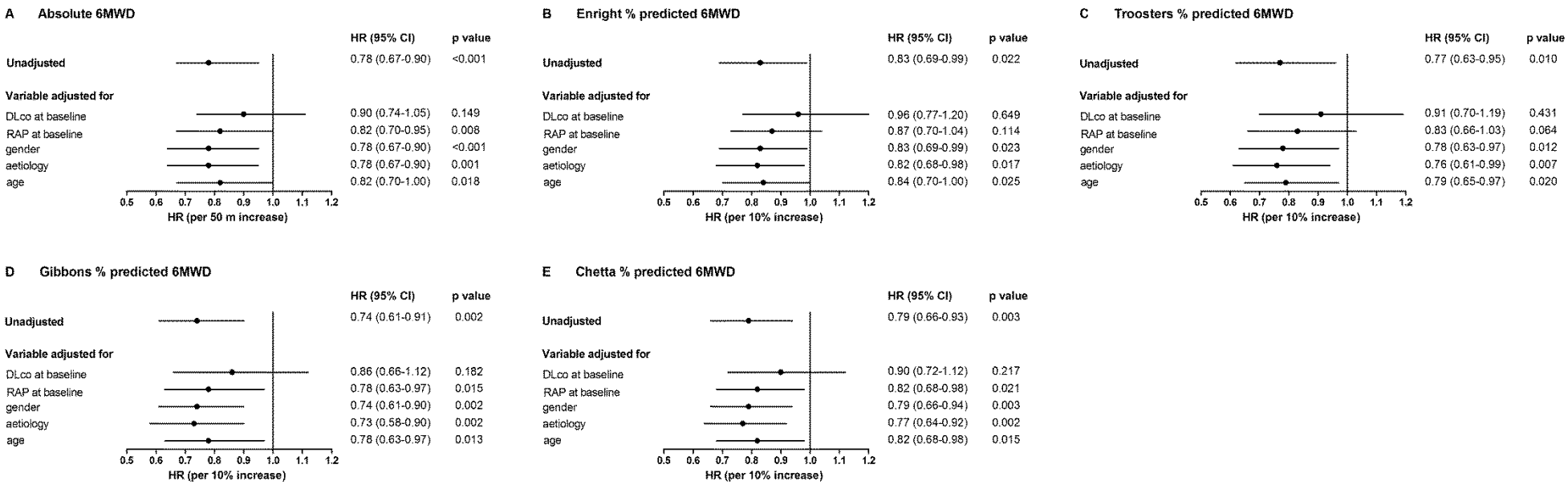
Age and % predicted DLco at baseline, absolute and % predicted 6MWD at baseline and on treatment, and WHO FC on treatment predicted all-cause mortality in univariate Cox proportional hazards analysis (table 5.3). The predictive value of absolute and % predicted 6MWD at baseline remained significant after adjusting for age, gender, aetiology but not % predicted DLco in bivariate Cox proportional hazards analysis (figure 5.1). Absolute 6MWD, and % predicted 6MWD by Gibbons and Chetta equations predicted mortality after adjusting for RAP, but % predicted 6MWD by Enright or Troosters equations did not. On treatment, absolute and % predicted 6MWD was predictive of mortality independently of WHO FC (except Enright equation), age, gender, aetiology, baseline % predicted DLco and RAP (figure 5.2). When combined with absolute or % predicted 6MWD on treatment, % predicted DLco at baseline no longer predicted mortality.

Table 5.3. Univariate Cox proportional hazards analysis

	Hazard ratio	95% CI	p value
Age at diagnosis, per decade increase	1.43	1.11-1.86	0.003
Gender			
Female	0.61	0.32-1.19	0.126
Male (reference)	–	–	–
Lung function at baseline			
FEV_1, per 10% predicted increase	1.09	0.91-1.31	0.383
FVC, per 10 % predicted increase	1.07	0.91-1.26	0.426
DLco, per 10 % predicted increase	0.75	0.63-0.90	0.002
WHO FC at baseline			
I/II	0.60	0.18-2.02	0.296
III	0.63	0.24-1.67	0.259
IV (reference)	–	–	–
WHO FC on treatment			
I/II	0.13	0.04-0.43	<0.001
III	0.28	0.09-0.89	0.003
IV (reference)	–	–	–
Aetiology			
CTDPAH	1.63	0.87-3.06	0.171
IPAH (reference)	–	–	–
Haemodynamics at baseline			
RAP, per 5 mmHg increase	1.19	0.90-1.58	0.136
mPAP, per 5 mmHg increase	1.01	0.91-1.12	0.797
CI, per 1 l/min/m^2 increase	0.83	0.49-1.39	0.487
PVR, per 1 Wood unit increase	1.01	0.96-1.06	0.672
S_vO_2, per 5% increase	0.93	0.73-1.19	0.601
6MWD at baseline (n=130)			
Absolute 6MWD, per 50 m increase	0.78	0.67-0.95	<0.001
% predicted 6MWD, per 10% increase			
Enright	0.83	0.69-0.99	0.022
Troosters	0.77	0.62-0.96	0.010
Gibbons	0.74	0.61-0.90	0.002
Chetta	0.79	0.66-0.94	0.003
6MWD on treatment (n=110)			
Absolute 6MWD, per 50 m increase	0.74	0.64-0.90	0.001
% predicted 6MWD, per 10% increase			
Enright	0.75	0.61-0.93	0.009
Troosters	0.68	0.53-0.89	0.003
Gibbons	0.67	0.53-0.85	0.001
Chetta	0.72	0.59-0.88	0.002

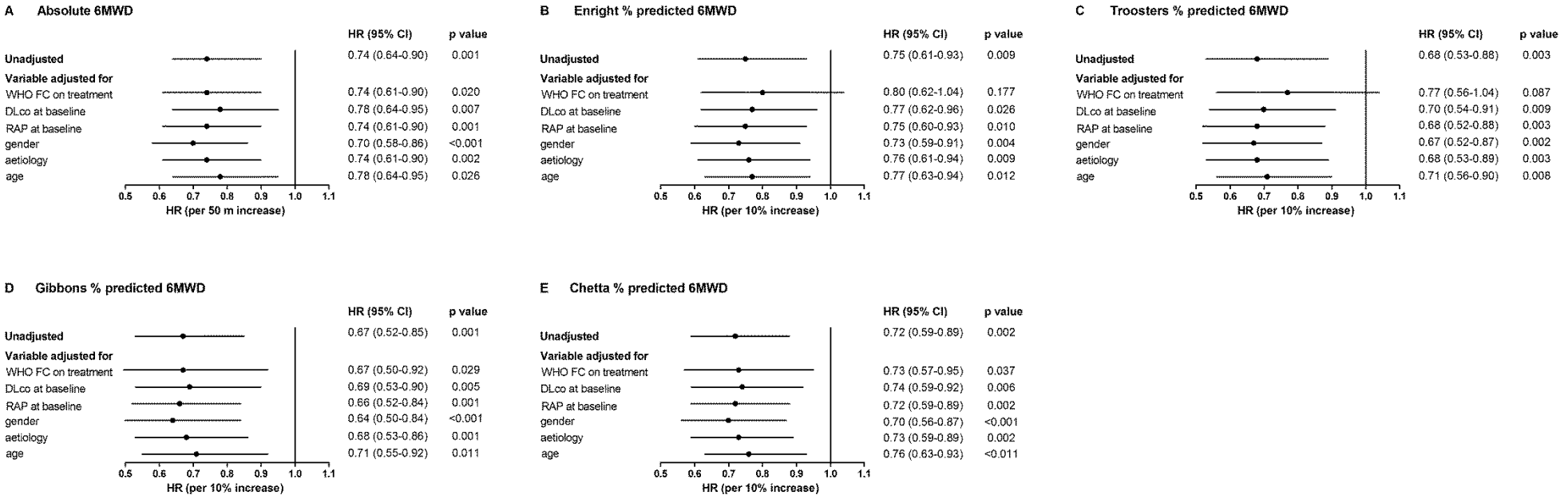
See table 5.2 for abbreviations

Figure 5.1. Bivariate Cox proportional hazards models of absolute and % predicted 6MWD by four different reference equations at baseline



DLco: diffusing capacity for carbon monoxide; RAP: right atrial pressure.
The unadjusted and adjusted hazard ratios (HR) for 6MWD are indicated by closed circles and the 95% CI by error bars.

Figure 5.2. Bivariate Cox proportional hazards models of absolute and % predicted 6MWD by four different reference equations on treatment

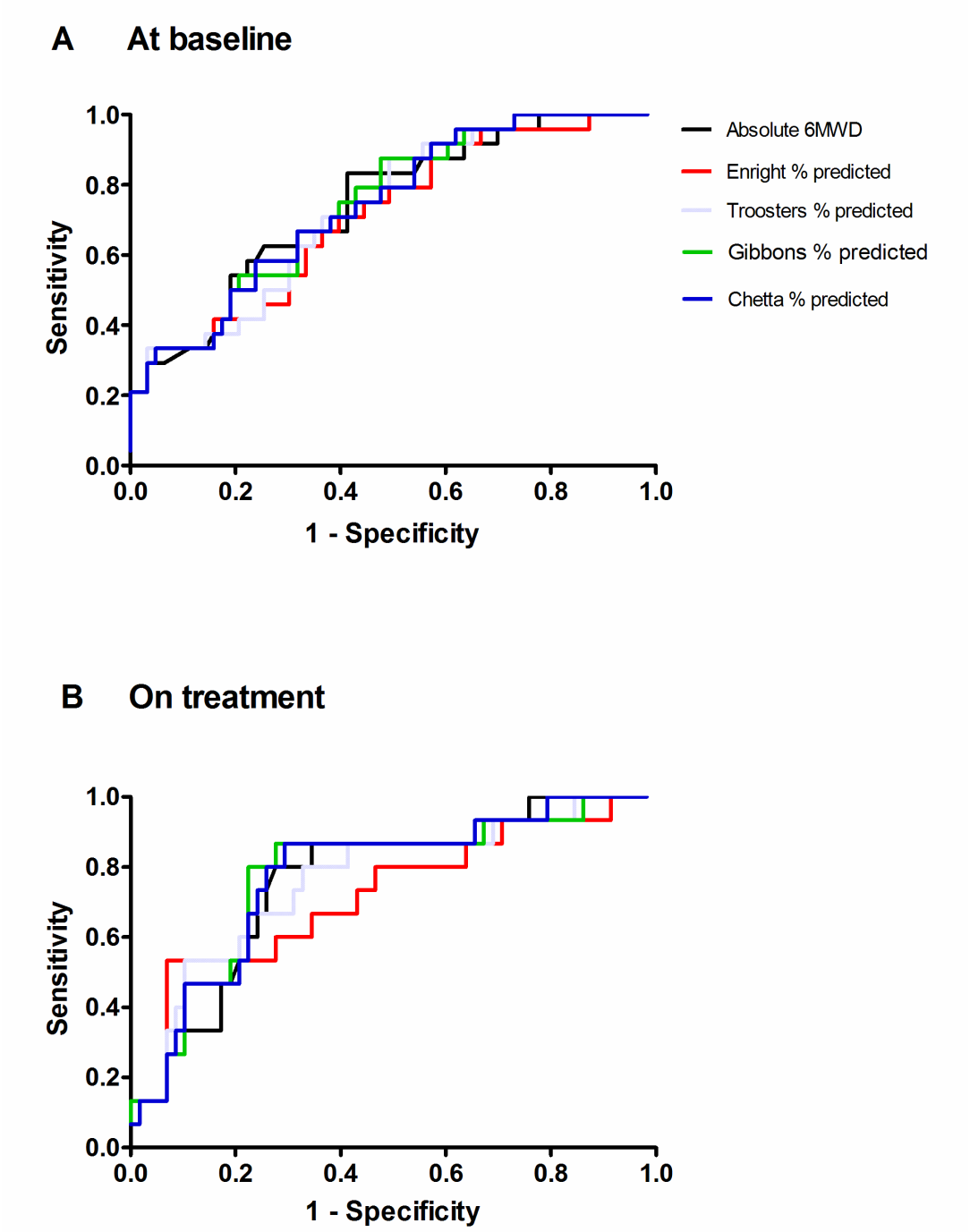


DLco: diffusing capacity for carbon monoxide; RAP: right atrial pressure; WHO FC: World Health Organisation functional class.
The unadjusted and adjusted hazard ratios (HR) for 6MWD are indicated by closed circles and the 95% CI by error bars.

5.4.4 Relative strength of mortality prediction

ROC curves of absolute 6MWD and % predicted 6MWD by each of four reference equations at baseline and on treatment are shown in figure 5.3. There were no significant differences in AUC of absolute versus each expression of % predicted 6MWD for predicting 2-year mortality at baseline or on treatment (table 5.4). In addition, there were no significant differences in AUC among % predicted 6MWD by the four reference equations. The optimal thresholds of absolute 6MWD and each expression of % predicted 6MWD at baseline and on treatment for predicting 2-year mortality had modest sensitivity and specificity. The thresholds of absolute 6MWD and % predicted 6MWD by equations from Troosters et al [66], Gibbons et al [65] and Chetta et al [63] on treatment had slightly higher sensitivity and specificity than those at baseline, but the respective differences in AUC between baseline and treatment did not reach statistical significance.

Figure 5.3. ROC curves of absolute and % predicted 6MWD by four different reference equations in predicting 2-year mortality



6MWD: six-minute walk distance; ROC: receiver operating characteristics. All p>0.05 when area under ROC curves is compared among expressions of 6MWD at baseline and on treatment, respectively, by a paired z test.

Table 5.4. ROC analysis comparing the ability of absolute and % predicted 6MWD to predict 2-year mortality

	ROC curve area (95% CI)	Optimal threshold	Sensitivity/specificity	p value
At baseline				
Absolute 6MWD	0.74 (0.63-0.86)	<295 m	0.83/0.59	<0.001
% predicted 6MWD				
Enright	0.71 (0.59-0.83)	<51%	0.75/0.56	0.002
Troosters	0.73 (0.62-0.85)	<43%	0.79/0.57	<0.001
Gibbons	0.75 (0.64-0.85)	<43%	0.75/0.60	<0.001
Chetta	0.74 (0.63-0.85)	<53%	0.75/0.57	<0.001
On treatment				
Absolute 6MWD	0.77 (0.64-0.90)	<296 m	0.87/0.66	0.002
% predicted 6MWD				
Enright	0.72 (0.56-0.87)	<53%	0.67/0.66	0.009
Troosters	0.76 (0.63-0.90)	<45%	0.80/0.67	0.002
Gibbons	0.78 (0.64-0.91)	<44%	0.87/0.72	0.001
Chetta	0.78 (0.65-0.91)	<53%	0.87/0.71	0.001

6MWD: six-minute walk distance; ROC: receiver-operating characteristic.

5.5 Discussion

This study addresses an important clinical issue regarding the role of % predicted 6MWD in the management of PAH. The data analysis showed that % predicted 6MWD at baseline derived from currently available reference equations predicts all-cause mortality, but its prognostic ability is not superior to that of absolute 6MWD despite adjusting for physiological inter-subject variance. In addition, there is no difference in the prognostic ability between each of the four studied reference equations. Assuming that the long-term prognosis of patients improves with PAH-specific therapy, 6MWD on treatment may be a stronger predictor of mortality than that at baseline, and hence the difference in the relative prognostic strength between absolute and % predicted 6MWD may be more apparent on treatment. However, this is not supported by the results of this analysis. % predicted 6MWD was not more predictive of mortality than absolute 6MWD on treatment.

% predicted 6MWD calculated from Troosters and Gibbons equations was slightly lower than that from Enright and Chetta equations. This disparity is due to differences in the 6MWT protocol used in the studies, such as the length of corridor, type and frequency of encouragement and the number of practice walks. A 50-m corridor was used by Troosters et al [66] and the best of 4 walks by Gibbons et al [65], which resulted in higher predicted absolute 6MWD. The equations were also derived from healthy adults in different age groups. Patients younger than 40 years of age were included by Chetta et al [63] and Gibbons et al [65] only. This highlights the importance of giving consideration to the characteristics of the study population and the 6MWT protocol used in deriving the reference equation when applying predicted values. The American Thoracic Society guidelines on 6MWT recognise that % predicted 6MWD is under-utilised due to a lack of optimal reference equations [49]. Only 40-66% of physiological inter-subject variance of 6MWD is explained by the currently available equations. Therefore, there are other factors influencing the performance at the 6MWT that have not been identified or accounted for. This may partly explain the failure to demonstrate the superiority of % predicted

6MWD by any of the four reference equations to absolute 6MWD in predicting mortality.

There are other potential explanations for the negative findings in this study. It might be possible for patients with low or high 6MWD to dominate the analysis, thereby obscuring any prognostic difference between absolute and % predicted 6MWD. However, this was not the case as the 6MWD data were normally distributed with few patients at either end of the range. The use of all-cause mortality rather than disease-specific mortality may also be relevant as this could shift the comparison of prognostic strength in favour of absolute 6MWD. For example, consider the case of a 40-year old male with severe PAH (height 186 cm, weight 90 kg) and an 80-year old female with mild PAH (height 157 cm, weight 60 kg) both of whom walk the same 6MWD of 300 m and survive for 5 years. This walk distance corresponds to 40% and 75% predicted, using Enright equation respectively, reflecting more severe disease in the male. The 40-year old male is more likely to die from PAH whereas the 80-year old female is more likely to die from other causes as she approaches the natural limit of life expectancy. In this scenario, absolute 6MWD is the better predictor of all-cause mortality as it has automatically incorporated the influence of age on mortality, with age being the most important physiological determinant of 6MWD. Conversely, % predicted 6MWD would be expected to perform better at predicting disease-specific mortality. All-cause mortality was used in this study to avoid the subjectivity associated with disease-specific mortality.

The fact that there is a considerable amount of unexplained physiological variance in 6MWD raises concerns about its reliability as an outcome measure in PAH. Some clinicians propose that 6MWD should be used instead to set a treatment goal in individual patients based on their starting parameters, where inter-subject variance is not as relevant. To set an appropriate goal, the knowledge of predicted 6MWD in an individual is essential. Therefore, despite having no added prognostic information, % predicted 6MWD would help clinicians to ascertain the degree of improvement achievable should a goal-oriented treatment strategy be adopted.

The study has a number of limitations. Firstly, selection bias cannot be excluded as this is a retrospective study and gaps due to missing data are unavoidable. 14 (10%) patients with no 6MWD data at baseline or within 12 months of starting treatment were excluded from the analysis. Secondly, patients were recruited over a relatively long period of time during which treatment approaches had evolved. This may have led to differences in prognosis of patients diagnosed in the earlier years compared to those diagnosed more recently. However, this is unlikely to have affected the main outcome of the analysis, namely the comparison between absolute and % predicted 6MWD. Finally, the reference equations were derived from healthy populations with differing age groups and hence no single equation was perfectly applicable to the study patients.

5.6 Conclusions

% predicted 6MWD may help clinicians quantify the functional impact of disease on an individual, but its prognostic ability is not superior to that of absolute 6MWD. In addition, the prognostic ability of currently available reference equations is similar. To explore the role of % predicted 6MWD in the management of PAH further, efforts should be made to develop more robust reference equations using a standardised 6MWT protocol and sampling healthy adults from more representative populations, particularly in relation to age.

6 PREDICTING SURVIVAL IN PULMONARY ARTERIAL HYPERTENSION IN THE UNITED KINGDOM

6.1 Summary

Rationale: Prognostic equations in PAH have been developed from contemporary cohorts in the United States and France. It is not known if these would perform as well in the UK as a locally derived scoring scheme.

Aims: To develop a UK risk score from a well-defined Scottish PAH cohort and to validate its prognostic performance against other published equations in a second independent UK PAH cohort.

Methods: Baseline mortality predictors identified by multivariate Cox analysis in 182 incident PAH patients were used to derive the Scottish Composite Score (SCS). Its prognostic performance in an independent UK PAH cohort was compared with the French Registry and PHC Registry equations using the BS.

Results: The SCS based on age, gender, aetiology, RAP, CO and 6MWD predicted survival in the validation cohort (hazard ratio 1.7 per point increase, $p<0.001$) and provided further prognostic stratification in WHO FC III patients (hazard ratio 1.8 per point increase, $p<0.001$). It was more accurate than the French Registry equation in predicting 1-year survival (BS: 0.092 versus 0.146, $p=0.001$) and 2-year survival (0.131 versus 0.255, $p<0.001$). There was no significant difference in BS between the SCS and PHC Registry equation.

Conclusions: The SCS predicts survival and can be used to supplement WHO FC in prognostication. It may perform better in UK populations than prognostic equations derived from other registry studies.

6.2 Introduction

Pulmonary arterial hypertension remains an incurable disease associated with high morbidity and mortality despite expansion of effective pharmacological therapy in the last decade [2]. As accurate prognostication is an integral part of disease management, considerable efforts have been made to identify prognostic factors and develop algorithms to predict patient outcome for clinical use. The first prognostic equation based on pulmonary haemodynamics (RAP, mPAP and CI at diagnosis) was derived from the NIH Registry study of 194 patients with primary pulmonary hypertension (now classified as idiopathic, heritable and anorexigen-associated PAH) from 32 centres across the United States two decades ago before the advent of PAH-specific therapy [1]. It has become obsolete but continues to be used as a benchmark for patient outcome without targeted therapeutic intervention. Survival analysis of a contemporary PAH cohort from the PHC Registry treated in a single centre in Chicago including demographics and functional measures identified the same haemodynamic variables as the NIH equation, and these were used to derive a new equation based on a similar methodological approach [72]. Another prognostic equation based on gender, 6MWD and CO has been developed from the French Registry including incident and prevalent patients diagnosed up to 3 years prior to study entry [73;140]. These two contemporary equations only apply to patients with idiopathic, familial and anorexigen-associated PAH whereas the equation developed from REVEAL included all patients in WHO group I PAH [71]. Due to its large sample size, a greater number of variables were able to be incorporated in the prediction model compared with the French Registry and PHC Registry equations (13 versus 3 variables).

These contemporary prognostic algorithms utilise baseline variables to calculate a predicted probability of survival at a certain time point during follow-up. Validation data on these equations published so far support their predictive value. The REVEAL equation and its simplified risk score (a 22-point scoring system) have been shown to have good discriminatory ability in a prospective PAH cohort of newly diagnosed patients [177]. When the PHC Registry and French

Registry equations were applied to a prospective cohort of PAH patients followed up in four randomised controlled clinical trials and their extension studies, there was good agreement between predicted and observed survival [141]. As these equations were developed from patient populations in the United States and France with differing demographics, healthcare systems and treatment approaches to the UK, they may not perform as well in UK PAH populations as a locally derived scoring scheme. The aims of this study were to develop a UK risk score to predict prognosis in PAH from a well-defined cohort in Scotland, and to validate its prognostic performance against other published prognostic equations in an independent UK PAH cohort.

6.3 Methods

6.3.1 Study design

A retrospective cohort of incident and treatment naive patients diagnosed with WHO group I PAH in the SPVU between November 2000 and September 2009 were used to derive the SCS. Exclusion criteria were PAH associated with congenital heart disease (CHD-PAH), long-term CCB responders, PCWP >15mmHg and significant lung disease (defined by lung function). The diagnosis of PAH was based on RHC in accordance with contemporary guidelines [2;145]. Patients were subsequently treated with conventional therapy (long-term warfarin, diuretics or supplemental oxygen) and PAH-specific monotherapy (prostacyclin analogues, phosphodiesterase-5 inhibitors or endothelin receptor antagonists). Sequential combination therapy was initiated as clinically indicated. Baseline data on demographics, RHC, lung function, WHO FC, 6MWD, NT-proBNP and CAMPHOR score recorded within 3 months of diagnostic RHC and prior to starting PAH-specific therapy were collected. The variables found to be independent mortality predictors in multivariate Cox analysis were included in the SCS. Weighted points were assigned to the threshold values of each variable identified by exploratory analysis. The SCS was obtained by summation of the points scored for each variable and finalised prior to validation in a cohort of incident and treatment naive idiopathic and heritable PAH patients treated in

the Pulmonary Vascular Disease Unit at Papworth Hospital (Cambridge) between January 2001 and December 2009. Patients with significant lung disease (defined by lung function), raised PCWP and long-term CCB responders were excluded. Baseline data were collected to apply the SCS, French Registry and PHC Registry equations. The ability of the SCS to predict survival over time and stratify patients into prognostic groups in the whole cohort and in WHO FC III patients was tested. The French Registry and PHC Registry equations were used to compute survival estimates at 1 and 2 years post-diagnosis. Their predictive accuracy was compared with that of the SCS using the BS. Patient consent was considered unnecessary by the local research ethics committee in the respective institutions.

6.3.2 Statistical analysis

Statistical analysis was performed using Statview version 5.0.1 (SAS Institute, Cary, NC, USA) and Graphpad Prism version 5.00 (Graphpad Software, La Jolla, CA, USA). Continuous variables were checked for normality using D'Agostino and Pearson omnibus normality test. Normally distributed variables are shown as mean ± SD and non-normally distributed variables are shown as median (IQR). Categorical variables are presented as number (%). Variables were compared using unpaired Student's t test, Mann-Whitney U test or Chi-squared test depending on data type and distribution.

Univariate Cox proportional hazards analysis was used to identify baseline mortality predictors in the derivation cohort. Survival time was calculated from the date of RHC to the date of death or data cut-off (1st May 2010). Patients who received lung transplantation or were lost to follow-up were censored on the date of procedure or last clinical contact. Significant continuous variables were transformed into categorised variables using threshold values identified in exploratory Cox analysis (see Appendix 3) and were assessed with their corresponding indicator variable coding for missing data ("missing" and "not missing") in a multivariate Cox model using a backward selection procedure. The use of indicator variables was to allow inclusion of patients with missing

data in multivariate analyses. None of the indicator variables for missing data were predictive of survival. Gender was not significant in the univariate Cox analysis, but was entered in the multivariate model as its prognostic significance has been confirmed in previous studies. Aetiology was divided into 2 subgroups: connective tissue disease associated pulmonary arterial hypertension due to systemic sclerosis (CTDPAH-SSc) and WHO group I PAH others versus idiopathic, heritable, anorexigen-associated and CTDPAH non-SSc (reference subgroup). Anorexigen-associated PAH was included in the reference subgroup as these patients are regarded as having similar outcomes to those with idiopathic and heritable PAH. "WHO group I PAH others" included patients with portopulmonary hypertension, human immunodeficiency virus and pulmonary veno-occlusive disease. Variables with $p<0.1$ were retained in the final multivariate Cox model. Weighted points were assigned to each variable subgroup according to their adjusted hazard ratio in the final model, for example, 0 point was assigned to the reference subgroup with an adjusted hazard ratio of 1. For the other subgroups, 1 point was assigned if the adjusted hazard ratio was 2, 2 points if the adjusted hazard ratio was 3 and 3 points if the adjusted hazard ratio was 4.

Kaplan Meier analysis was used to estimate survival rates and comparison between groups was made by the log-rank test for trend. Cox analysis was used to evaluate the ability of the SCS to predict survival in the validation cohort. The BS was used to assess the accuracy of the SCS, French Registry and PHC Registry equations in predicting 1- and 2-year survival in the validation cohort allowing for censoring. It measures the mean squared deviation of predicted probability from the actual outcome. A BS of 0 indicates perfect prediction. A BS of 0.25 indicates that the prediction is equivalent to the outcome occurring by chance alone. A lower BS indicates higher prediction accuracy. To compare the performance of the SCS with other equations, a point estimate of difference in BS between SCS and each equation and its 95% confidence interval was obtained from 200,000 bootstrap re-samples. A difference in BS of >0.02 was considered clinically relevant. A p value<0.05 was defined as statistically significant.

6.4 Results

6.4.1 Derivation of the Scottish Composite Score

Characteristics of the derivation cohort are summarised in table 6.1. 10% of patients received prostacyclin analogues (intravenous epoprostenol 7%, inhaled iloprost 2%, subcutaneous treprostinil 1%), 47% phosphodiesterase-5 inhibitors (sildenafil 45% and tadalafil 2%) and 44% endothelin receptor antagonists (bosentan 29%, sitaxentan 7% and ambrisentan 8%). After a median follow-up period of 25 months (range 0.1 to 113 months), 81 patients died from all causes, two patients received lung transplantation, one patient was lost to follow-up and two patients transferred care to another centre. The survival estimates for the whole cohort were 79% at 1 year, 68% at 2 years and 57% at 3 years. Out of the 17 baseline variables considered in the univariate Cox model, age, aetiology, % predicted DLco, RAP, CO, WHO FC, 6MWD, NT-proBNP and CAMPHOR score were significant mortality predictors (table 6.2). When these variables were categorised into 2 to 4 subgroups and assessed along with gender in a multivariate Cox model, age at diagnosis, gender, aetiology, RAP, CO and 6MWD were retained as independent mortality predictors and were used to construct the SCS (0 to 8) (table 6.3).

Table 6.1. Baseline characteristics of the derivation cohort

	All patients	Aetiology reference subgroup	p value
Patient number, n	182	125	-
Age, years	62 (48-73)	61 (45-71)	0.592
Female, n (%)	125 (69)	81 (65)	0.477
Aetiology, n (%)			
Idiopathic PAH	97 (53)	97 (78)	–
Heritable PAH	2 (1)	2 (2)	–
CTDPAH			–
SSc associated	33 (18)	–	–
non-SSc associated	26 (14)	26 (21)	–
WHO group I PAH others			
PoPH	15 (8)	–	–
HIV	1 (0.5)	–	–
PVOD	8 (4)	–	–
Lung function, % predicted			
FEV_1	87 (75-98)[*]	86±17[**]	0.605
FVC	99 (86-112)[*]	98±19[**]	0.628
DLco	42 (28-60)[‡]	44 (28-63)[‡‡]	0.645
Pulmonary haemodynamics			
RAP, mmHg	7 (4-11)	6 (4-10)	0.421
mPAP, mmHg	47 (39-55)	48 (40-58)	0.459
CO, l/min	3.6 (2.9-4.7)	3.6 (2.9-4.7)	0.929
PVR, Wood units	10.8 (7.4-15.5)	11.2 (7.6-15.7)	0.596
SvO_2, %	64 (57-70)	65 (58-70)	0.789
WHO FC, n (%)			
I and II	25 (14)	19 (15)	0.899
III	131 (72)	87 (70)	–
IV	26 (14)	19 (15)	–
6MWD, m	260±109[¶]	273±110[¶¶]	0.342
NT-proBNP, pg/ml	1026 (298-2637)[§]	972 (316-2258)[§§]	0.856
CAMPHOR	38 (27-57)[#]	40±18[##]	0.933
Year of diagnosis			
Prior to 2005	51 (28)	36 (29)	0.882
2005 onwards	131 (72)	89 (71)	–

Data are expressed as mean ± SD, median (IQR) or n (%). 6MWD: six-minute walk distance; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; CO: cardiac output; CTDPAH: connective tissue disease associated pulmonary arterial hypertension; DLco: carbon monoxide diffusing capacity; FC: functional class; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; HIV: human immunodeficiency virus; mPAP: mean pulmonary artery pressure; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PoPH: portopulmonary hypertension; PVOD: pulmonary veno-occlusive disease; PVR: pulmonary vascular resistance; RAP: right atrial pressure; S_vO_2: mixed venous saturation; SSc: systemic sclerosis; WHO FC: World Health Organisation. [*]: n=171; [‡]: n=158; [¶]: n=177; [§]: n=96; [#]: n=76; [**]: n=117; [‡‡]: n=108; [¶¶]: n=122; [§§]: n=63; [##]: n=51.

Table 6.2. Univariate Cox proportional hazards analysis in the derivation cohort

	Hazard ratio	95% CI	p value
Age at diagnosis, per decade increase	1.32	1.12-1.57	0.010
Gender			
Female	0.76	0.47-1.23	0.260
Male (reference)			
Aetiology subgroups	-	-	0.001
WHO group I PAH others[†]	3.21	1.79-5.76	<0.001
CTDPAH-SSc	1.74	0.98-3.09	0.057
CTDPAH non-SSc	1.29	0.67-2.50	0.445
Idiopathic or heritable PAH (reference)			
Lung function			
FEV_1, per 10% increase	0.07	0.00-3.02	0.165
FVC, per 10% increase	0.32	0.02-5.32	0.892
DLco, per 10% increase	0.80	0.70-0.91	<0.001
Pulmonary haemodynamics			
RAP, per 5 mmHg increase	1.30	1.08-1.57	<0.001
mPAP, per 5 mmHg increase	1.03	0.96-1.10	0.480
S_vO_2 , per 5% increase	0.95	0.83-1.09	0.464
PVR, per 5 Wood units increase	1.13	0.95-1.33	0.170
CO, per l/min increase	0.83	0.68-1.01	0.058
WHO FC	-	-	0.010
I and II	0.30	0.12-0.72	0.007
III	0.49	0.28-0.85	0.010
IV (reference)			
6MWD, per 100m increase	0.61	0.50-0.67	<0.001
NT-proBNP, per 200 pg/ml increase	1.02	1.00-1.04	0.036
CAMPHOR, per 5 points increase	1.16	1.04-1.29	0.008
Year of diagnosis			
2005 onwards	1.15	0.71-1.86	0.572
2000-2005 (reference)			

6MWD: six-minute walk distance; CO: cardiac output; CTDPAH: connective tissue disease associated pulmonary arterial hypertension; DLco: carbon monoxide diffusing capacity; FEV_1: forced expiratory volume in one second; FC: functional class; FVC: forced vital capacity; mPAP: mean pulmonary artery pressure; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; RAP: right atrial pressure; S_vO_2: mixed venous saturation; SSc: systemic sclerosis; WHO: World Health Organisation.
[†]: includes portopulmonary hypertension, human immunodeficiency virus, pulmonary veno-occlusive disease.

Table 6.3. Multivariate mortality predictors and derivation of the Scottish Composite Score

Variables	Categories	HR (95% CI)	Points
Age, years	≥ 70	2.20 (1.35 - 3.61)	1
	<70 (reference)	-	0
Gender	Male	2.01 (1.15 - 3.51)	1
	Female (reference)	-	0
Aetiology	CTDPAH-SSc or group I PAH others[†]	2.33 (1.42 - 3.82)	1
	Idiopathic, heritable, anorexigen-associated PAH or CTDPAH non-SSc (reference)	-	0
6MWD, m	<50	5.04 (2.33 - 10.89)	3
	50-149	3.45 (1.48 - 8.05)	2
	150-299	2.48 (1.28 - 4.79)	1
	≥ 300 (reference)	-	0
RAP, mmHg	≥ 10	1.50 (0.90 - 2.49)	1
	<10 (reference)	-	0
CO, l/min	<3	2.18 (1.32 - 3.61)	1
	≥ 3 (reference)	-	0

6MWD: six-minute walk distance; CO: cardiac output; CTDPAH: connective tissue disease associated pulmonary arterial hypertension; HR: hazard ratio; PAH: pulmonary arterial hypertension; RAP: right atrial pressure; SSc: systemic sclerosis.
[†]: includes portopulmonary hypertension, human immunodeficiency virus and pulmonary veno-occlusive disease.

6.4.2 Validation of the Scottish Composite Score

Out of 119 patients in the validation cohort, 99 patients had complete data to calculate the SCS (table 6.4). 38 patients died and four received lung transplantation during follow-up (median 29 months, range 0.3 to 102 months). The 1-year, 2-year and 3-year survival estimates for patients in whom SCS was available were 87%, 74% and 68%, which was not significantly different from those in whom SCS was not available (log-rank p=0.196). The risk of death increased with the SCS (hazard ratio 1.7 per point increase, 95% CI 1.4-2.1, p<0.001). When patients were stratified into 3 SCS risk groups - "high risk" = 4-8 (n=23), "intermediate risk" = 2-3 (n=44) and "low risk" = 0-1 (n=32), there was a significant difference in survival between groups (p<0.001 by log-rank test for trend) (figure 6.1). The SCS further stratified WHO FC III patients (hazard ratio 1.8 per point increase, 95% CI 1.4-2.4, p<0.001) (n=66, 30 deaths). When these patients were stratified into 3 SCS risk groups ("high risk" = 4-8, "intermediate risk" = 2-3, "low risk" = 0-1), there was a significant difference in survival between groups (p<0.001 by log-rank test for trend) (figure 6.2).

6.4.3 Comparison of prognostic equations

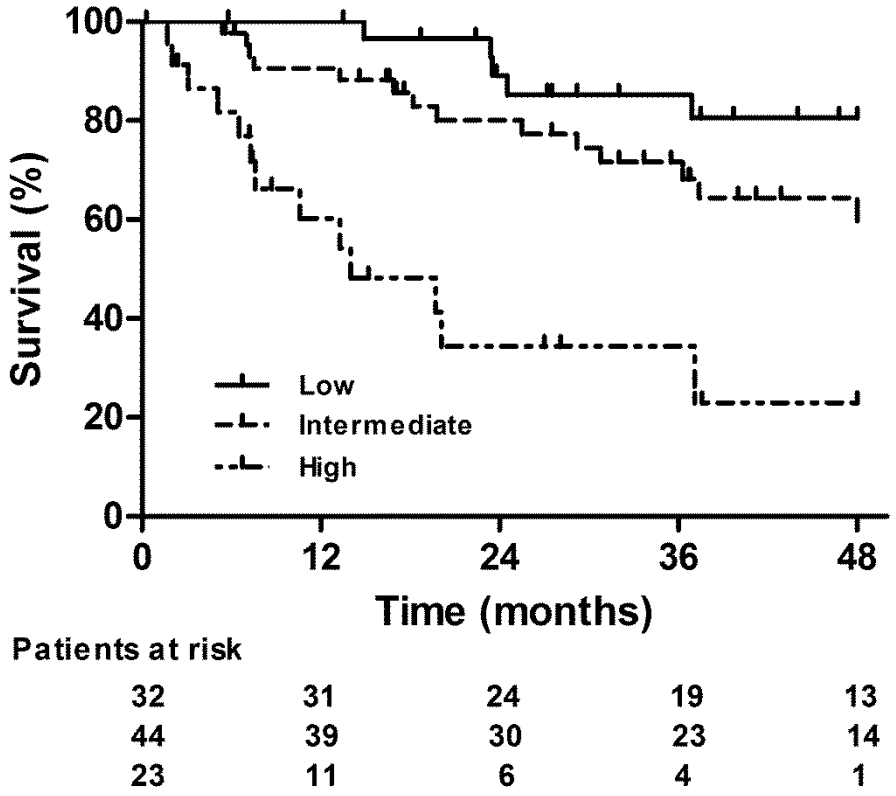
When the SCS, French Registry and PHC Registry equations were applied to the validation cohort to predict 1-and 2-year survival, the SCS had a lower BS than both equations, but only the comparison with the French Registry equation reached statistical significance (table 6.5).

Table 6.4. Characteristics of the validation cohort

	Derivation cohort	Validation cohort	p value
Patient number, n	182	99	-
Age, years	62 (48-73)	53 (42-69)	0.010
Female, n (%)	125 (69)	72 (73)	0.479
Aetiology, n (%)			
Idiopathic	97 (53)	96 (97)	–
Heritable PAH	2 (1)	3 (3)	–
CTDPAH		–	–
SSc associated	33 (18)	–	–
non-SSc associated	26 (14)	–	–
WHO group I PAH others			
PoPH	15 (8)	–	–
HIV	1 (0.5)	–	–
PVOD	8 (4)	–	–
Lung function, % predicted			
FEV_1	87 (75-98)[*]	84±15	0.038
FVC	99 (86-112)[*]	97±17	0.094
DLco	42 (28-60)[‡]	59±22[‡‡]	<0.001
Haemodynamics			
RAP, mmHg	7 (4-11)	9±6	0.013
mPAP, mmHg	47 (39-55)	50 (42-59)	0.016
CO, L/min	3.6 (2.9-4.7)	3.2 (2.6-3.8)	0.002
PVR, Wood units	10.8 (7.4-15.5)	13.3±5.4	0.011
S_vO_2, %	64 (57-70)	63±8	0.889
WHO FC, n (%)			
I and II	25 (14)	18 (18)	0.572
III	131 (72)	66 (67)	–
IV	26 (14)	15 (15)	–
6MWD, metres	260±109[¶]	267±121	0.669
NTproBNP, pg/mL	1026 (298-2637)[§]	2029 (330-4407)[**]	0.134
CAMPHOR	38 (27-57)[#]	38 (26-57)[§§]	0.804

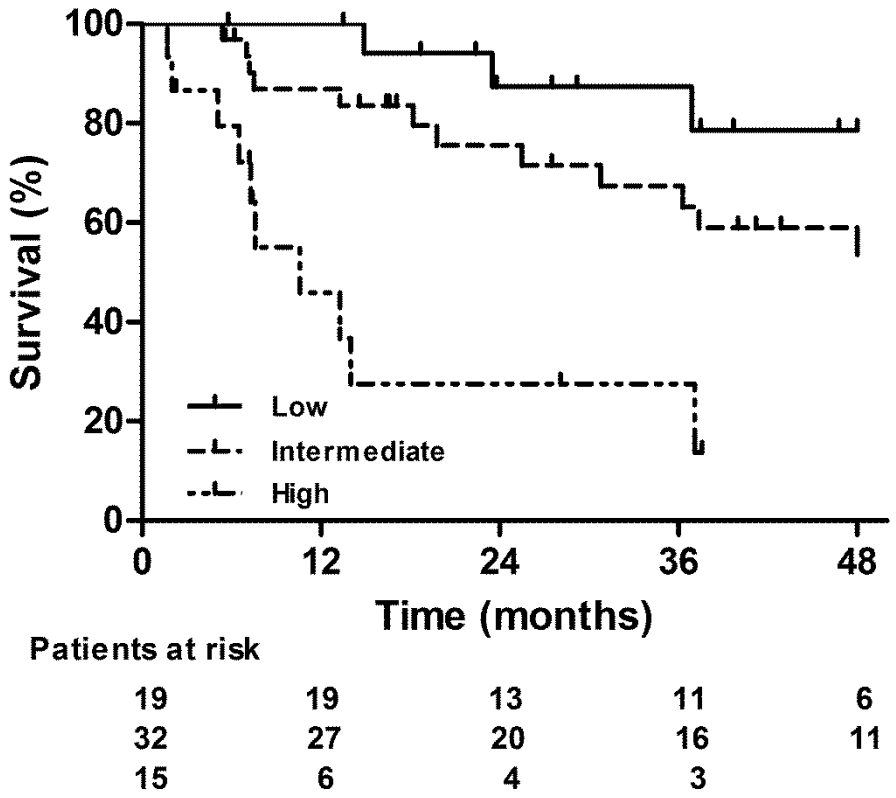
Data are expressed as mean ± SD, median (IQR) or n (%).
See table 6.1 for abbreviations.
[*]:n=171; [‡]:n=158; [¶]: n=177; [§]: n=96; [#]: n=76; [‡‡]: n=81; [**]: n=45; [§§]: n=49.

Figure 6.1. Kaplan Meier survival estimates of the validation cohort stratified by three Scottish Composite Score risk groups



p<0.001 by log-rank test for trend

Figure 6.2. Kaplan Meier survival estimates of WHO FC III patients in the validation cohort stratified by three Scottish Composite Score risk groups



WHO FC: World Health Organisation functional class. p<0.001 by log-rank test for trend

Table 6.5. Comparison of predictive accuracy between the Scottish Composite Score and other published prognostic equations

	Brier Score	95% CI	p value
Predicting 1-year survival			
French Registry equation	0.146	0.120 to 0.175	–
PHC Registry equation	0.111	0.060 to 0.168	–
SCS	0.092	0.058 to 0.130	–
Difference: SCS – French Registry equation	-0.055	-0.083 to -0.024	0.001
Difference: SCS – PHC Registry equation	-0.019	-0.059 to 0.017	0.328
Predicting 2-year survival			
French Registry equation	0.255	0.210 to 0.302	–
PHC Registry equation	0.189	0.133 to 0.250	–
SCS	0.131	0.091 to 0.175	–
Difference: SCS – French Registry equation	-0.124	-0.176 to -0.070	<0.001
Difference: SCS – PHC Registry equation	-0.058	-0.122 to -0.002	0.060

PHC: Pulmonary Hypertension Connection; SCS: Scottish Composite Score. A BS of 0 indicates perfect prediction and 0.25 indicates a prediction equivalent to random chance

6.5 DISCUSSION

The present study is the first to describe the derivation and validation of a risk score to predict prognosis in incident PAH patients based on UK data. The data analysis showed that the SCS, a 8-point simple scoring system based on age, gender, aetiology, RAP, CO and 6MWD at diagnosis, can be used to categorise patients into prognostic groups and provide further risk stratification in WHO FC III patients. When tested in an independent UK PAH cohort, the predictive accuracy of the SCS was comparable to that of the PHC Registry equation and may be greater than the French Registry equation. The prognostic significance of gender and aetiology demonstrated by previous studies was also confirmed. These findings add to the growing body of literature on prognostication in PAH and provide further insights into the use of contemporary prognostic equations derived from other registry studies.

The predictive value of the French Registry, PHC Registry and REVEAL equations has been demonstrated in respective validation studies. However their applicability in other patient populations would be influenced by factors such as study population characteristics, treatment pathways and statistical methodology (table 6.6). The SCS was designed as a simple point-based risk score to stratify patients into prognostic groups whereas the other equations were developed to compute predicted survival probabilities at certain time points post-diagnosis. Both SCS derivation and validation cohorts were treated in single tertiary centres and this would ensure complete data capture and uniformity of care. On the contrary, the French Registry and REVEAL included national cohorts from selected centres, which may lead to selection bias, variations in clinical practice and patient outcome. The SCS was derived from a strictly incident and treatment naive patient cohort whereas French Registry, PHC Registry and REVEAL equations from mixed incident and prevalent cohorts. The inclusion of prevalent patients would introduce survivor bias as these patients have survived long enough to be recruited in the study and would have a better prognosis than those who die early from severe disease or lack of response to PAH therapy. This was clearly demonstrated by comparing survival

between incident and prevalent cohorts in the French Registry study [140]. In order to remove this survivor bias, authors of the French Registry equation estimated survival from time of diagnosis as opposed to time of enrolment and only considered patients to be at risk from their time of study entry. This adjustment for the time delay between diagnosis and study entry in prevalent patients was not adopted in the derivation of the PHC Registry or REVEAL equations. The study period of the PHC Registry spanned both pre- and post- modern treatment eras. Changes in patient prognosis over this period due to treatment advances are probable and this may have affected the survival analysis. These factors, in addition to variations in health care systems and treatment approaches, would impact on the performance of these prognostic algorithms in different clinical settings.

It is well recognised that patient outcome differs according to PAH aetiology. Patients with CHD-PAH were excluded from this study as they have a distinct haemodynamic profile and natural history from other PAH patients [178]. Pulmonary arterial hypertension associated with SSc is known to confer a worse prognosis than idiopathic PAH despite a similar degree of haemodynamic derangement for reasons that are not fully understood [74;179]. When PAH associated with different types of connective tissue disease including mixed connective tissue disease, overlap syndromes and rheumatoid arthritis were grouped together and compared with CTDPAH-SSc in the derivation cohort, CTDPAH-SSc conferred a worse prognosis. This would be consistent with the findings from a UK-wide epidemiology study [88]. Patients with idiopathic, heritable, anorexigen-associated PAH are regarded as having similar outcomes as they share clinical and pathophysiological features [180]. Hence the grouping of aetiologies in the SCS would be consistent with findings from previous studies. An alternative approach to developing the risk score is to focus on patients with idiopathic, heritable, anorexigen-associated PAH and exclude other subgroups especially those with SSc given its worse prognosis. As interaction between aetiology and other mortality predictors may alter their prognostic characteristics, a given predictor may perform differently in different aetiology subgroups. While focusing on one homogeneous patient group may allow the

development of a more accurate model prediction for that particular group, a single risk score applicable to all PAH patients would be simpler to use and more readily adopted in routine clinical practice. The premise of this study was to develop a simple composite risk score that could be used to risk stratify patients regardless of PAH aetiology while taking in account the influence of aetiology on survival.

There is also increasing evidence to support a gender difference in patient outcome and this is confirmed by the present study. More females are affected by PAH than males, but they have a better prognosis [73;181]. Recent analysis of patients enrolled in REVEAL showed that males had a higher mPAP and RAP at diagnosis and those aged ≥60 years had lower survival rates compared with females aged ≥60 years [182]. Sex hormones are thought to play a role, but the precise mechanisms remain to be elucidated. The individual prognostic value of other component variables in the SCS has also been demonstrated in other studies (age [71;72;181], 6MWD [44;46;58-60;71;73], RAP [43;44;59;60;71;138], CO [73]).

Algorithms advising on the initial choice of drugs and the use of combination therapy advocated by current guidelines on PAH management are primarily based on WHO FC as its prognostic value has been firmly established [2;5;173]. However, WHO FC may not be a sufficiently reliable measure of functional status as wide variation in clinicians' assessment has been reported [183]. The differences between WHO FC I or II and IV are clear-cut, but WHO FC III encompasses patients with a wide range of functional capacity. The results of this study showed that the SCS could provide further risk stratification in WHO FC III patients and hence supplement WHO FC in clinical assessment. Variables measured during follow-up may be more predictive of long-term outcome than those measured at baseline evaluation as they would capture the impact of treatment response and disease progression on survival. A recent study has confirmed the prognostic impact of changes in outcome variables during the course of disease and demonstrated the importance of incorporating them into risk assessment [184], but there are currently no published prediction tools specifically developed to address this issue. We propose that the SCS could be

used to assess the initial risk of mortality in the one to two year time horizons with a view to develop a risk score incorporating changes in mortality predictors over time for repeated use during follow-up.

This study has several limitations. Missing data were unavoidable due to the retrospective nature of the study. As measurements of NT-proBNP and CAMPHOR score were not introduced into clinical practice until 2004, there were fewer data compared to other baseline variables which may have introduced bias against them in multivariate survival analyses despite statistical adjustment. The derivation cohort consisted of patients with heterogeneous aetiologies of PAH with relatively small numbers in some subgroups. However, this simply reflects the relative incidence of different PAH aetiologies in a real-life clinical cohort. The data on vasoreactivity status at diagnosis were incomplete, so the effect of a positive vasodilatory response on survival could not be assessed. Only one patient demonstrated sustained CCB response and was excluded from the study. This is based on the finding that long-term CCB responders have a better prognosis than non-CCB responders [185]. As the number is small, the bias associated with this exclusion would be insignificant. There were no patients with rarer causes of WHO group I PAH such as schistosomiasis or chronic haemolytic anaemia in either the derivation or validation cohort, and only patients with idiopathic and heritable PAH were included in the validation cohort. Hence the performance of the SCS in other PAH subgroups is still to be validated. 17% of patients in the validation cohort did not have all the required variables to calculate the SCS. It was not possible to determine how the SCS would perform in these patients compared to other equations, but there was no difference in survival between patients with and without the SCS. Heart rate and SBP were measured in each patient at diagnosis but not recorded in the validation database and hence unavailable for this analysis. The REVEAL equation could not be included in the comparison as the systematic omission of these data would lead to bias in its performance. This is a limitation of database analysis that does not reflect the potential value of an assessment tool in clinical practice

Table 6.6. Prognostic algorithms in pulmonary arterial hypertension

	Study population	Recruitment period	Variables	End-point	Validation
French Registry equation [73;140]	idiopathic, familial and anorexigen-associated PAH n: 190, 29% Incident and 71% prevalent cases	2002-2003, follow-up time 3 years for all patients	6MWD, gender, CO	survival up to 3 years post-diagnosis	Prospective validation in PAH cohorts from clinical trials
Pulmonary Hypertension Connection Registry equation [72]	idiopathic, familial and anorexigen-associated PAH n: 282, incident and prevalent cases	1991-2007, median follow-up time 3.9 years, IQR 1.7 to 7.8 years, maximum follow-up time 16.6 years	RAP, mPAP, CI	survival at number of years post-diagnosis	Prospective validation in PAH cohorts from clinical trials
REVEAL Registry equation and risk score [71;177]	WHO group I PAH n: 2716, 14% incident and 86% prevalent cases	2006 onwards, mean follow-up time 1.4 years, range 0 to 2 years	age, aetiology, gender, renal insufficiency, SBP, HR, WHO FC, 6MWD, BNP or NT-proBNP, RAP, PVR, presence of pericardial effusion, % predicted DLco	1-year survival	Prospective validation in newly diagnosed PAH patients from REVEAL
Scottish Composite Score	WHO group I PAH (except for CHD-PAH) n: 182, all incident cases	2000-2009, median follow-up time 2.1 years, range 3 days to 9.4 years	age, aetiology, gender, 6MWD, RAP and CO	survival at number of years post-diagnosis	Retrospective validation in an independent UK idiopathic and heritable PAH cohort

6MWD: six-minute walk distance; BNP: brain natriuretic peptide; CHD-PAH: congenital heart disease associated pulmonary arterial hypertension; CI: cardiac index; CO: cardiac output; DLco: carbon monoxide diffusing capacity; HR: heart rate; mPAP: mean pulmonary artery pressure; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; RAP: right atrial pressure; SBP: systolic blood pressure; WHO FC: World Health Organisation functional class.

6.6 Conclusions

The SCS is a simple multidimensional risk score combining the impact of demographics, pulmonary haemodynamics and functional status on survival in incident PAH patients. When validated in an independent UK idiopathic and heritable PAH cohort, it correlated with mortality and provided further risk stratification in WHO FC III patients. It may perform better in UK populations than prognostic equations derived from other registry studies, but further validation in wider PAH populations is required before firm conclusions on its clinical utility can be made.

7 MAJOR FINDINGS AND CONCLUSIONS

The work in this thesis was undertaken to evaluate the use of novel non-invasive exercise variables and prognostic algorithms as alternative outcome measures to 6MWD in PH.

The ability of non-invasive haemodynamic measurements and isotime ventilatory variables measured at submaximal constant-load exercise to predict treatment response was investigated in patients with precapillary PH. The major findings were:-

- The IGR method could be used to measure PBF and SV non-invasively at rest and during exercise with good intersession reproducibility.
- Resting and submaximal exercise IGR PBF and SV correlate with conventional makers of disease severity including WHO FC, 6MWD, NT-proBNP and CAMPHOR score.
- Resting and submaximal exercise IGR PBF and SV could be used to detect treatment response to PAH-specific therapy, and may be more sensitive than 6MWD in detecting the effects of therapy in fitter patients walking >450 m.
- Isotime ventilatory indices of cardiac function (VO_2, VO_2/HR) and ventilatory efficiency (V_E/VCO_2, $P_{ET}CO_2$) at submaximal constant-load exercise were less useful in detecting treatment response.

The ability of $P_{ET}CO_2$ during the 6MWT to predict treatment response was investigated, but the study was under-powered to draw any conclusions from the negative results.

- No significant changes were detected in ventilatory variables during the 6MWT after new PAH-specific therapy except for a trended improvement in end-of-walk RER.
- Therapy-induced changes in ventilatory indices of cardiac function (end-of-walk VO_2 and VO_2/HR) and ventilatory efficiency (end-of-walk V_E/VCO_2 and

$P_{ET}CO_{2\ nadir}$) correlated with those in 6MWD. This is in keeping with the mechanisms behind improved 6MWD with therapy.

The role of % predicted 6MWD in the management of PAH was explored in patients with IPAH and CTDPAH.

- % predicted 6MWD adjusts for physiological inter-subject variance, but is not superior to absolute 6MWD in predicting survival in PAH at baseline or on treatment.

A novel composite risk score was developed from a Scottish cohort of incident and treatment-naive PAH patients and then validated externally in an independent UK IPAH cohort.

- The SCS combines prognostic information from age, gender, PAH aetiology, RAP, CO and 6MWD at baseline.
- It could be used to stratify WHO group I PAH patients into risk groups in the one to two year time horizons and supplement WHO FC in prognostic assessment.
- It may perform better in UK populations than other published prognostic equations derived from registry studies conducted in France and the United States.

In conclusion, the aim of this thesis, namely to evaluate the use of novel non-invasive outcome measures in PH, was achieved. The work on IGR haemodynamic measurements and the SCS was most fruitful and could be developed in several directions. The use of submaximal exercise IGR haemodynamic measurements should be explored further in patient groups where the ability of 6MWD to detect clinical change is most limited, such as CTDPAH patients with comorbid musculoskeletal or pulmonary abnormalities and fitter patients with early disease. Resting IGR haemodynamic measurements may be useful as outcome measures in WHO FC IV patients who are unable to perform exercise tests. Simultaneous measurement of VO_2 and SV during

submaximal exercise may help to identify patients with impaired peripheral oxygen extraction who may benefit from exercise training. The use of $P_{ET}CO_2$ during 6MWT as an outcome measure warrants further investigation and power calculation for future studies could be performed using data from this work. Results on the prognostic performance of the SCS are encouraging but need to be confirmed in patients with CTDPAH and other WHO group I aetiologies. It would be of great interest to develop a composite risk score based on changes in outcome variables during the course of disease to capture the prognostic impact of disease progression and PAH-specific therapy.

APPENDIX 1. 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.

APPENDIX 2. CAMPHOR QUESTIONNAIRE

CAMPHOR

Cambridge Pulmonary Hypertension Outcome Review

Please read this carefully

On the following pages you will find some statements that have been made by people who have Pulmonary Arterial Hypertension.

Please read each statement carefully.
We would like you to put a tick in the box ☑ next to **'Yes'** if you feel it applies to you and a tick in the box ☑ next to **'No'** if it does not

Please choose the response that applies best to you

at the moment

Symptoms

Please read each statement carefully and decide whether it applies to you ***at the moment***

1. My stamina levels are low — Yes ❒ No ❒
2. I have to rest during the day — Yes ❒ No ❒
3. I feel worn out — Yes ❒ No ❒
4. I get tired very quickly — Yes ❒ No ❒
5. I'm tired all the time — Yes ❒ No ❒
6. I feel very weak — Yes ❒ No ❒
7. I feel completely exhausted — Yes ❒ No ❒
8. I want to sit down all the time — Yes ❒ No ❒
9. I soon run out of energy — Yes ❒ No ❒

10. Everything is an effort — Yes ❒ No ❒

11. I get out of breath when I stand up — Yes ❒ No ❒

12. When I talk I get out of breath — Yes ❒ No ❒

Please read each statement carefully and decide whether it applies to you ***at the moment***

13. When I walk I get out of breath — Yes ❒ No ❒

14. I get breathless if I bend — Yes ❒ No ❒

15. I get breathless going up one step — Yes ❒ No ❒

16. I get breathless walking up a slight slope — Yes ❒ No ❒

17. I get breathless without doing anything — Yes ❒ No ❒

18. I get breathless climbing a flight of stairs — Yes ❒ No ❒

19. I have mood swings — Yes ❒

No ❒

20. I get very down — Yes ❒ No ❒

21. I seldom feel happy — Yes ❒ No ❒

Please read each statement carefully and decide whether it applies to you ***at the moment***

22. I've forgotten what it's like to enjoy myself — Yes ❒ No ❒

23. I feel hopeless — Yes ❒ No ❒

24. It does get me down — Yes ❒ No ❒

25. I often feel anxious — Yes ❒ No ❒

Activities

Please put a tick in the box ☑ under the response which best describes your abilities at the moment**.** Please respond to all 15 statements.

Please describe your ability without the use of aids or assistance. However, do describe your ability taking into account oxygen if you use it.

Please mark only one box.	Able to do on own **without** difficulty	Able to do on own **with** difficulty	Unable to do on own
1. Cut your toenails	☐	☐	☐
2. Have an all over wash	☐	☐	☐
3. Get dressed	☐	☐	☐
4. Walk around inside the house (not including climbing stairs)	☐	☐	☐
5. Walk short distances on level ground	☐	☐	☐
6. Walk longer distances on level ground	☐	☐	☐
7. Walk up a slight incline	☐	☐	☐
8. Climb a flight of stairs	☐	☐	☐
9. Bend down to pick objects up from the floor	☐	☐	☐
10. Stand for a short time	☐	☐	☐
11. Stand for a long time	☐	☐	☐
12. Lift heavy items	☐	☐	☐
13. Carry heavy items	☐	☐	☐
14. Do light jobs around the house or garden	☐	☐	☐
15. Do heavy jobs around the house or garden	☐	☐	☐

Quality of Life

Please read each statement carefully and put a tick ☑ next to the response that applies best to you ***at the moment***

1. I have to talk very quietly — True ☐ Not True ☐

2. I can't stay away from home — True ☐ Not True ☐

3. I've lost interest in food — True ☐ Not True ☐

4. I can't put energy into my close relationships — True ☐ Not True ☐

5. Walking for pleasure is out of the question — True ☐ Not True ☐

6. My condition puts a strain on my close relationships — True ☐ Not True ☐

7. I feel very isolated — True ☐ Not True ☐

8. I can’t do things on the spur of the moment — True ☐ Not True ☐

9. I feel vulnerable when I'm on my own — True ☐ Not True ☐

10. It feels like my body has let me down — True ☐ Not True ☐

11. I feel as if I'm not in control of my life — True ☐ Not True ☐

12. I feel dependent on other people — True ☐ Not True ☐

Please remember to put a tick in ***only one*** of the alternative responses for each of the statements

13. .Sometimes it's too much effort to speak — True ☐ Not True ☐

14. I feel as if I am a burden to people — True ☐ Not True ☐

15. Travelling distances is a problem — True ☐ Not True ☐

16. I don't like to be seen like this — True ☐ Not True ☐

17. I feel that I'm losing my role in life — True ☐ Not True ☐

18. I worry that I neglect people close to me — True ☐ Not True ☐

Please read each statement carefully and decide whether it applies to you ***at the moment***

19. I feel guilty asking for help — True ☐ Not True ☐

20. My condition limits the places I can go — True ☐ Not True ☐

21. I dislike having to rely on other people — True ☐ Not True ☐

22. I don't want to talk to anybody — True ☐ Not True ☐

23. I feel as if I let people down — True ☐ Not True ☐

24. I am reluctant to leave the house — True ☐ Not True ☐

25. I'm unable to join in activities with my family and friends

True ☐

Not True ☐

Thank you for taking the trouble to fill in this questionnaire.

Please check all the pages to make sure that you have answered every statement

APPENDIX 3. TRANSFORMATION OF CONTINUOUS UNIVARIATE MORTALITY PREDICTORS IN THE DEVELOPMENT OF THE SCS

Meaningful thresholds of age at diagnosis, RAP, CO, % predicted DLco, 6MWD, NT-proBNP and CAMPHOR score were identified by exploratory analysis. Each variable was dichotomised into two subgroups starting from a low threshold and analysed in a univariate Cox model as a categorised variable. This was carried out repeatedly with the threshold increasing by fixed increments. Care was taken to ensure there were sufficient patient numbers and deaths in each subgroup. The threshold that yielded the most significant hazard ratio was used to categorise the variable into two subgroups. If more than one meaningful threshold was identified, they were used to divide the variable into three or more subgroups. Different combinations were explored and the one that yielded the best separation of hazard ratios between subgroups was used to categorise the variable. The analyses on each variable are outlined as follows.

Age - Step 1 (table A.1)

The subgroup above the threshold was defined as the reference group. Thresholds of 40 years, 50 years, 60 years and 70 years yielded significant results. Age 70 years was chosen to be the upper threshold and further dichotomised Cox analysis was performed in patients aged <70 years to identify a lower threshold.

Age - Step 2 (table A.2)

No further significant thresholds were identified for age <70 years. So age was categorised into 2 subgroups, ≥70 years and <70 years.

Table A.1 Dichotomised univariate Cox analysis of age (Step 1)

Threshold (years)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<30	8/174 (3/78)	0.80	0.702
<40	25/157 (6/75)	0.42	0.039
<50	48/134 (14/67)	0.55	0.043
<60	83/99 (28/53)	0.57	0.016
<70	126/56 (47/34)	0.41	<0.001
<80	176/6 (79/2)	0.80	0.752

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.2. Dichotomised univariate Cox analysis of age in patients <70 years (Step 2)

Threshold (years)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<30	8/118 (3/44)	1.07	0.913
<40	26/100 (7/40)	0.62	0.237
<50	48/78 (14/33)	0.76	0.380
<60	83/43 (28/19)	0.87	0.647

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

RAP - Step 1 (table A.3)

The subgroup above the threshold was defined as the reference group. Thresholds of 5 mmHg and 10 mmHg yielded significant results. RAP 10 mmHg was chosen to be the upper threshold and further dichotomised Cox analysis was performed in patients with RAP <10mmHg to identify a lower threshold.

RAP - Step 2 (table A.4)

No further significant thresholds were identified for RAP <10mmHg. So RAP was categorised into 2 subgroups, ≥10 mmHg and< 10 mmHg.

Table A.3. Dichotomised univariate Cox analysis of RAP (Step 1)

Threshold (mmHg)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<5	53/129 (17/64)	0.53	0.021
<10	126/56 (50/31)	0.51	0.003
<15	156/26 (68/13)	0.65	0.161
<20	177/5 (80/1)	1.94	0.511

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.4. Dichotomised univariate Cox analysis of RAP in patients with RAP<10 mmHg (Step 2)

Threshold (mmHg)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<1	13/113 (4/46)	0.88	0.807
<2	21/105 (7/43)	0.94	0.885
<3	35/91 (11/39)	0.72	0.334
<4	40/86 (12/38)	0.67	0.221
<5	53/73 (17/33)	0.67	0.175
<6	72/54 (25/25)	0.65	0.123
<7	84/42 (32/18)	0.70	0.219
<8	101/25 (38/12)	0.60	0.125

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

CO - Step 1 (table A.5)

The subgroup above the threshold was defined as the reference group. Threshold of 3.0 l/min yielded the most significant result. It was chosen to be the lower threshold and further dichotomised Cox analysis was performed in patients with CO >3.0 l/min to identify an upper threshold.

CO - Step 2 (table A.6)

No further significant thresholds were identified for CO >3.0 l/min. So CO was categorised into 2 subgroups, ≥3.0 l/min and <3.0 l/min.

Table A.5. Dichotomised univariate Cox analysis of CO (Step 1)

Threshold (l/min)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<2.0	4/176 (2/77)	0.87	0.844
<2.5	17/163 (11/68)	1.77	0.080
<3.0	46/134 (28/51)	2.05	0.002
<3.5	82/98 (43/36)	1.59	0.042
<4.0	108/72 (53/26)	1.44	0.132
<4.5	126/54 (59/20)	1.36	0.233
<5.0	142/38 (64/15)	1.20	0.519
<5.5	161/19 (70/9)	0.99	0.981
<6.0	168/12 (75/4)	1.52	0.415

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.6. Dichotomised univariate Cox analysis of CO in patients with CO ≥ 3.0 l/min (Step 2)

Threshold (l/min)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<3.5	36/98 (15/36)	1.10	0.760
<4.0	62/72 (25/26)	1.06	0.844
<4.5	80/54 (31/20)	1.04	0.882
<5.0	96/38 (36/15)	0.92	0.792
<5.5	115/19 (42/9)	0.77	0.487
<6.0	122/12 (47/4)	1.23	0.691

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

% predicted DLco - Step 1 (table A.7)

The subgroup above the threshold was defined as the reference group. Thresholds of 30%, 35%, 40%, 45%, 50%, 55% and 60% yielded similarly significant results. DLco 60% predicted was chosen to be the upper threshold and further dichotomised Cox analyses were performed in patients with DLco <60% to identify a lower threshold.

% predicted DLco - Step 2 (table A.8)

Thresholds of 30% and 40% yielded similarly significant results. So % predicted DLco was categorised into 3 subgroups differently, ≥60, 40-59, <40 or ≥60, 30-59, <30. Both were assessed in the multivariate model sequentially.

Table A.7. Dichotomised univariate Cox analysis of % predicted DLco (Step 1)

Threshold (% predicted)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<20	13/145 (8/58)	2.19	0.038
<25	28/130 (15/51)	1.91	0.028
<30	42/116 (23/43)	2.27	0.002
<35	58/100 (32/34)	2.11	0.003
<40	70/88 (37/29)	2.35	<0.001
<45	87/71 (44/22)	2.35	0.001
<50	95/63 (48/18)	2.60	<0.001
<55	108/50 (51/15)	2.32	0.005
<60	115/43 (55/11)	2.52	0.006
<65	127/31 (57/9)	1.89	0.082
<70	137/21 (59/7)	1.52	0.294
<75	144/14 (62/4)	2.03	0.170
<80	149/9 (64/2)	2.46	0.210

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.8. Dichotomised univariate Cox analysis of % predicted DLco in patients with DLco <60% predicted (Step 2)

Threshold (% predicted)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<20	13/102 (8/47))	1.76	0.142
<25	28/87 (15/40)	1.50	0.183
<30	42/73 (23/32)	1.79	0.035
<35	58/57 (32/23)	1.63	0.076
<40	70/45 (37/18)	1.85	0.034
<45	87/28 (44/11)	1.79	0.086
<50	95/20 (48/7)	2.14	0.061
<55	108/7 (51/4)	1.47	0.461

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

6MWD - Step 1 (table A.9)

The subgroup above the threshold was defined as the reference group. Thresholds of 50 m,100 m, 150 m, 200 m, 250 m and 300 m yielded similarly significant results. A 6MWD of 300 m was chosen to be the upper threshold and further dichotomised Cox analyses were performed in patients with 6MWD <300 m to identify a lower threshold.

6MWD - Step 2 (table A.10)

Thresholds of 150 m and 50 m yielded the most significant results.

6MWD - Step 3 (figure A.1)

Dividing 6MWD into 4 subgroups, <50 m, 50-149 m, 150-299 m and ≥300 m and using ≥300 m subgroup as the reference group, there was an incremental increase in hazard ratio with each subgroup. The 6MWD <50 m subgroup had the worst outcome and the ≥300 m subgroup the best outcome.

Table A.9. Dichotomised univariate Cox analysis of 6MWD (Step 1)

Threshold (m)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<50	27/175 (21/66)	4.02	<0.001
<100	36/166 (27/60)	4.17	<0.001
<150	49/153 (36/51)	3.93	<0.001
<200	80/122 (49/38)	2.94	<0.001
<250	105/97 (63/24)	3.63	<0.001
<300	130/72 (73/14)	4.13	<0.001
<350	164/38 (79/8)	3.12	0.002
<400	188/14 (85/2)	3.74	0.066
<450	195/7 (86/1)	3.10	0.261

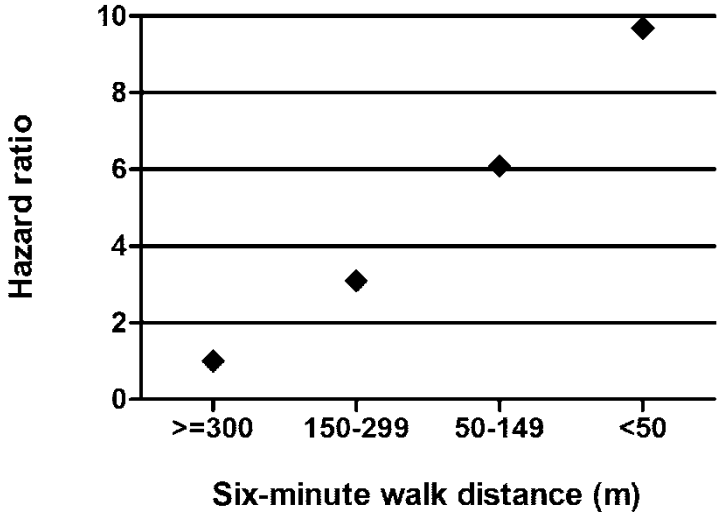
N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.10. Dichotomised univariate Cox analysis of 6MWD in patients with 6MWD <300 m (Step 2)

Threshold (m)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<50	27/103 (21/52)	2.76	<0.001
<100	36/94 (27/46)	2.43	<0.001
<150	49/81 (36/37)	2.52	<0.001
<200	80/50 (49/24)	1.74	0.026
<250	105/25 (63/10)	2.03	0.039

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Figure A.1. Distribution of hazard ratios among 6MWD subgroups



NT-proBNP - Step 1 (table A.11)

NT-proBNP was log transformed and thresholds of $10^{2.9}$ pg/ml, $10^{3.0}$ pg/ml, $10^{3.1}$ pg/ml, $10^{3.2}$ pg/ml and $10^{3.3}$ pg/ml yielded significant results. These thresholds corresponded to NT-proBNP 794 pg/ml, 1000 pg/ml, 1259 pg/ml, 1585 pg/ml and 1995 pg/ml respectively.

NT-proBNP - Step 2 (table A.12)

Dichotomised Cox analysis was repeated using thresholds at 100 pg/ml increments from 900 to 2000 pg/ml. The subgroup above the threshold was defined as the reference group. All yielded significant results.

NT-proBNP - Step 3 (table A.13)

Using 2000 pg/ml as an upper threshold, no lower threshold was identified.

NT-proBNP - Step 4 (table A.14)

Using 900 pg/ml as a lower threshold, no upper threshold was identified. So NT-proBNP was categorised into 2 subgroups, using thresholds at 100 pg/ml increments from 900 to 2000 pg/ml, each of which was tested in the multivariate model sequentially.

Table A.11. Dichotomised univariate Cox analysis of log NT-proBNP (Step 1)

Threshold (pg/ml)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
$<10^{2.0}$	7/19 (2/37)	0.47	0.294
$<10^{2.1}$	12/84 (4/35)	0.58	0.307
$<10^{2.2}$	14/82 (6/33)	0.83	0.672
$<10^{2.3}$	17/79 (7/32)	0.75	0.501
$<10^{2.4}$	20/76 (7/32)	0.61	0.232
$<10^{2.5}$	26/70 (8/31)	0.49	0.075
$<10^{2.6}$	29/67 (10/29)	0.59	0.148
$<10^{2.7}$	33/63 (12/27)	0.64	0.201
$<10^{2.8}$	35/61 (13/26)	0.70	0.305
$<10^{2.9}$	41/55 (13/26)	0.49	0.038
$<10^{3.0}$	48/48 (15/24)	0.44	0.013
$<10^{3.1}$	51/45 (15/24)	0.40	0.006
$<10^{3.2}$	58/38 (18/21)	0.42	0.007
$<10^{3.3}$	66/30 (22/17)	0.45	0.013
$<10^{3.4}$	70/26 (26/13)	0.61	0.148

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.12. Dichotomised univariate Cox analysis of NT-proBNP (Step 2)

Threshold (pg/ml)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<900	43/53 (13/26)	0.41	0.009
<1000	48/48 (15/24)	0.44	0.013
<1100	49/47 (15/24)	0.43	0.012
<1200	50/46 (15/24)	0.41	0.007
<1300	53/43 (16/23)	0.42	0.009
<1400	53/43 (16/23)	0.42	0.009
<1500	54/42 (17/22)	0.45	0.013
<1600	58/38 (18/21)	0.42	0.007
<1700	60/36 (20/19)	0.47	0.020
<1800	62/34 (20/19)	0.45	0.013
<1900	64/32 (21/18)	0.44	0.012
<2000	66/30 (22/17)	0.45	0.013
<2100	66/30 (22/17)	0.45	0.013

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.13. Dichotomised univariate Cox analysis of NT-proBNP in patients with NT-proBNP <2000 pg/ml (Step 3)

Threshold (pg/ml)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<900	43/23 (13/9)	0..49	0.114
<1000	48/18 (15/7)	0.56	0.225
<1100	49/17 (15/7)	0.55	0.204
<1200	50/16 (15/7)	0.42	0.118
<1300	53/13 (16/6)	0.52	0.182
<1400	53/13 (16/6)	0.52	0.182
<1500	54/12 (17/5)	0.57	0.289
<1600	58/8 (18/4)	0.46	0.168
<1700	60/6 (20/2)	0.76	0.717
<1800	62/4 (20/2)	0.62	0.514
<1900	64/2 (21/1)	0.54	0.550

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.14. Dichotomised univariate Cox analysis of NT-proBNP in patients with NT-proBNP ≥900 pg/ml (Step 4)

Threshold (pg/ml)	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<1000	5/48 (2/24)	0.85	0.830
<1100	6/47 (2/24)	0.80	0.762
<1200	7/46 (2/24)	0.57	0.446
<1300	10/43 (3/23)	0.69	0.540
<1400	10/43 (3/23)	0.69	0.540
<1500	11/42 (4/22)	0.78	0.645
<1600	15/38 (5/21)	0.62	0.342
<1700	17/36 (7/19)	0.81	0.640
<1800	19/34 (7/19)	0.73	0.482
<1900	21/32 (8/18)	0.70	0.404
<2000	23/30 (9/17)	0.70	0.401

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

CAMPHOR - Step 1 (table A.15)

The subgroup above the threshold was defined as the reference group. Threshold of 55 yielded the most significant result. It was chosen to be the upper threshold and further dichotomised Cox analysis was performed in patients with CAMPHOR <55 to identify a lower threshold.

CAMPHOR - Step 2 (table A.16)

No further significant thresholds were identified for CAMPHOR <55. So CAMPHOR was categorised into 2 subgroups, ≥55 and <55.

Table A.15. Dichotomised univariate Cox analysis of CAMPHOR score (Step 1)

Threshold	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<15	5/71 (1/28)	0.34	0.287
<20	9/67 (3/26)	0.70	0.556
<25	18/58 (5/24)	0.46	0.115
<30	23/53 (6/23)	0.48	0.112
<35	28/48 (8/21)	0.41	0.041
<40	39/37 (11/18)	0.38	0.016
<45	44/32 (13/16)	0.42	0.026
<50	48/28 (14/15)	0.61	0.098
<55	55/21 (16/13)	0.30	0.002
<60	62/14 (22/7)	0.43	0.060
<65	68/8 (25/4)	0.61	0.369

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

Table A.16. Dichotomised univariate Cox analysis of CAMPHOR score in patients with CAMPHOR <55 (Step 2)

Threshold	Total N_{above}/N_{below} (dead N_{above}/N_{below})	Hazard ratio	p value
<15	5/50 (1/15)	0.51	0.517
<20	9/46 (3/13)	1.07	0.918
<25	18/37 (5/11)	0.72	0.544
<30	23/32 (6/10)	0.76	0.601
<35	28/27 (8/8)	0.73	0.535
<40	39/16 (11/5)	0.72	0.542
<45	44/11 (13/3)	1.00	0.999

N_{above}: number of patients above the threshold ; N_{below}: number of patients below the threshold.

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