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Improving the Diagnosis and Assessment of Pulmonary Hypertension by Optimising Clinical Pathways

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DONALD HEATH SHEFFIELD
PULMONARY HYPERTENSION RESEARCH

To my children; Emily, Rory and Matilda.

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Declaration

This thesis has been written by Charlotte Durrington and represents the culmination of four years of work based at the Pulmonary Vascular Disease Unit, the Royal Hallamshire Hospital, Sheffield. The work on which this thesis is based is the candidates own. This thesis has not been submitted in candidature for any other degree, diploma or qualification.

Date …….. **Charlotte Durrington……**

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Synopsis

Pulmonary hypertension (PH) is a life limiting condition, however with the development of new management strategies and treatments overall survival has improved. Nevertheless, there are still improvements to be made in the assessment of patients with PH and enhancing clinical pathways to assist with screening of patients and ongoing monitoring to allow for early detection of deterioration prompting the need for escalation of treatments.

The first part of this thesis, examines the use of natriuretic peptides in field of PH. Examining N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and brain natriuretic peptide (BNP) in particular, both of which are incorporated into most risk stratification models in PH. An important limitation of these natriuretic peptides is the long laboratory processing time. Chapter 3 explores a point-of-care test (POCT) for NT-proBNP and BNP and examines its reliability. In addition, a real-world test of NT-proBNP's potential role in remote monitoring is examined by posting blood samples to a laboratory for analysis of NT-proBNP.

The second part of this thesis, chapter 4, investigates the impact of an integrated acute pulmonary embolism (PE) pathway, in a large tertiary pulmonary vascular disease referral centre upon diagnostic rates, disease severity and outcome of patients who go on to develop chronic thromboembolic pulmonary hypertension (CTEPH) following a PE.

In conclusion, utilising POCT for both NT-proBNP and BNP produced reliable results that were quicker and easier to process than laboratory samples. Furthermore, despite

a delay in processing by posting blood samples the NT-proBNP results were reliable highlighting the potential for use of this biomarker in the remote monitoring of patients with pulmonary arterial hypertension (PAH).

Since the incorporation of the integrated PE pathway, there have been increased population-based rates of both CTEPH diagnosis and pulmonary endarterectomy (PEA), identifying CTEPH patients earlier and with less severe disease. It was also demonstrated that the absence of major transient risk factors for PE and computed tomography (CT) features of PH at diagnosis predict the development of CTEPH.

Publications and Presentations to Learned Societies Arising from the Work Presented in this Thesis

Publications, publications in preparation and in submission

1. **Durrington C**, Hurdman JA, Elliot CA, Maclean R, Van Veen J, Saccullo G, De-Foneska D, Swift AJ, Smitha R, Hill C, Thomas S, Dwivedi K, Alabed S, Wild JM, Charalampopoulos A, Hameed A, Rothman AMK, Watson L, Hamilton N, Thompson AAR, Condliffe R, Kiely DG. Systematic pulmonary embolism follow-up increases diagnostic rates of chronic thromboembolic pulmonary hypertension and identifies less severe disease: results from the ASPIRE Registry. Eur Respir J. 2024 Mar 14;63(3):2300846. doi: 10.1183/13993003.00846-2023. PMID: 38302154; PMCID: PMC7615743 - *Published*
2. **Charlotte Durrington**, Christian Battersby, Laura Holt, Alexandra Fairman, Thomas Salisbury, Helena A. Turton, Lisa Watson, Ian Smith, Stefan Roman, Jenna Ablott, Chloe Roddis, Eleanor Oakes, Heather Wilshaw, Iain Woodrow, Iain Armstrong, Athanasios Charalampopoulos, Charlie Elliot, Abdul Hameed, Neil Hamilton, Judith Hurdman, Allan Lawrie, Jennifer T. Middleton, Hamza Zafar, Alex M. K. Rothman, Robin Condliffe, Rob Lewis, Rob Grange, David G Kiely, A. A. Roger Thompson. Assessing the reliability and stability of natriuretic peptide testing in pulmonary arterial hypertension and the potential for point of care testing and remote monitoring – *Currently Submitted*
3. Kiely DG, Hamilton N, Wood S, **Durrington C**, Exposto F, Muzwidzwa R, Raiteri L, Beaudet A, Muller A, Sauter R, Pillai N, Lawrie A; ASPIRE consortium. Risk assessment and real-world outcomes in chronic thromboembolic pulmonary hypertension: insights from a UK pulmonary hypertension referral service. BMJ Open. 2024 Jan 4;14(1):e080068. doi: 10.1136/bmjopen-2023-080068. PMID: 38176861; PMCID: PMC10773408 – *Published*
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Prizes

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Abbreviations

Aa	Amino acid
ABG	Arterial blood gas
AKL1	Activin A receptor II-like kinase 1
ASIG	Australian scleroderma interest group
BMPR2	Bone morphogenic protein receptor type II
BNP	Brain natriuretic peptide
BPA	Balloon pulmonary angiogram
cGMP	Cyclic guanosine monophosphate
CHD	Congenital heart defects
CI	Cardiac index
CMR	Cardiac magnetic resonance
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
COMPERA	Comparative, Prospective Registry of Newly Initiated Therapies
CT	Computerised tomography
CTD	Connective tissue disease
CTEPD	Chronic thromboembolic pulmonary disease
CTEPH	Chronic thromboembolic pulmonary hypertension
CTPA	Computerised tomography pulmonary angiography
DVT	Deep vein thrombosis
ECG	Electrocardiogram
ECHO	Echocardiogram
ENG	Endoglin
ERA	Endothelin receptor antagonists
ESC/ERS	European society of cardiology / European respiratory society
FEV1	Forced expired volume

FVC	Forced vital capacity
GC	Guanylyl cyclase
GMP	Cyclic guanosine monophosphate
GP	General Practitioner
HHT	Hereditary haemorrhagic telangiectasia
HPAH	Hereditary pulmonary arterial hypertension
HIV	Human immunodeficiency virus
HRCT	High resolution computed tomography
ILD	Interstital lung disease
IPF	Idiopathic pulmonary fibrosis
IPAH	Idiopathic pulmonary hypertension
ISWT	Incremental shuttle walk test
I.V	Intravenous
LAM	Lymphangioleiomyomatosis
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVF	Left ventricular failure
MAPK	Mitogen-activated protein kinase
mLAP	Mean left atrial pressure
mPAP	Mean pulmonary arterial pressure
MRI	Magnetic resonance imaging
NHS	National health service
NIV	Non-invasive ventilation
NPPB	Natriuretic peptide B
NPR	Natriuretic peptide receptor
NPR-C	Natriuretic peptide receptor type C
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
NYHA FC	New York Heart Association Functional Class
OHS	Obesity hypoventilation syndrome
OSA	Obstructive sleep apnoea

PA	Pulmonary artery
PAH	Pulmonary arterial hypertension
PAWP	Pulmonary arterial wedge pressure
PCH	Pulmonary capillary hemangiomatosis
PDE5 I	Phosphodiesterase type 5
PE	Pulmonary embolism
PEA	Pulmonary endarterectomy
PH	Pulmonary hypertension
PH-LH	Pulmonary hypertension – left heart disease
PIOPED	Prospective Investigation On Pulmonary Embolism Diagnosis
PKC	Protein kinase C
POCT	Point of care test
PRA	Prostacyclin receptor agonist
PVDU	Pulmonary vascular disease unit
PVOD	Pulmonary veno-occlusive disease
PVR	Pulmonary vascular resistance
QC	Quality control
RAP	Right atrial pressure
REVEAL	Registry to evaluate early & long-term PAH disease management
RHC	Right heart catheter
RV	Right ventricle
RVOTH	Right ventricular outflow tract hypertrophy
S.C	Subcutaneous
SGCS	soluble guanylate cyclase stimulator
sPAP	Systolic pulmonary arterial pressure
SSc	Systemic sclerosis
STH-ObS	STH Observation Study of PH, Cardiovascular and Respiratory Diseases
STH	Sheffield Teaching Hospitals

TGF	Transforming growth factor
TLco	Transfer factor of carbon monoxide
TTE	Transthoracic echocardiogram
UK	United Kingdom
VKA	Vitamin K antagonist
V/Q scans	Perfusion lung scintigraphy
VTE	Venous thromboembolism
WHO FC	World health organisation functional class
WU	Woods units
6MWT	6-minute walk test

Chapter 1: Introduction

Chapter 1 provides an overview of the topic of PH, describing the normal physiology of the pulmonary circulation, the pathophysiological classification of PH, and common investigations performed to aid the diagnosis of PH and the subtype. Treatment options and risk stratification are discussed with a focus on PAH. The aim of this M.D is to explore improved diagnosis and assessment of PH by optimising clinical pathways. This will be done by examining the utilisation of biomarkers in PH and by quantifying the development of CTEPH following PE. Therefore, the final parts of this chapter will describe the background literature surrounding these two sub-topics.

1.1 The normal pulmonary circulation

The role of the pulmonary circulation is primarily gas exchange. This occurs across the thin and highly permeable membranes of the alveolar capillaries. To allow this process to occur affectively, the pulmonary circulation, in health, pulmonary pressures must remain low, to allow the entire cardiac output (CO) of de-oxygenated blood to pass through the lungs via the pulmonary arteries, where it is oxygenated before being delivered back to the left heart and then dispersed into the systemic circulation. In normal adults at sea level, the normal mean pulmonary arterial pressure (mPAP) at rest is 14±3 mmHg. [1, 2] There are several factors that can elevate mPAP, for example in response to hypoxia when physiological pulmonary vasoconstriction occurs to maintain the ventilation-perfusion matching. Other influencing factors on increased mPAP include age and exercise. The latter, even in health, can cause mPAP to rise

above 30mmHg. [1, 3] However, compensatory mechanisms prevent mPAP from rising excessively by a corresponding fall in pulmonary vascular resistance (PVR). This is mediated by passive distension of a compliant system and active vasodilation mediated by nitric oxide. [4]

1.2 Pulmonary Hypertension

PH in its many forms is characterised by an elevation of pulmonary artery pressure. Understanding of PH has advanced significantly over the last 50 years. The first reported case of PAH was in 1891, when German physician Dr E. Romberg described at autopsy features of thickened pulmonary arteries with no heart or lung disease to explain the changes. This he termed ‘pulmonary vascular sclerosis’. [5] However, it was not until the right heart catheter (RHC) procedure was developed enough in the early 1950’s that a diagnosis of PH could be made with confidence. [6–8]

The first World Symposium on PH was held in 1973. PH was initially categorised in to two types; primary where no obvious other cause is identified, and secondary where a specific cause is found. [9] Nearly 50 years on the 6th World Symposium of PH was held in 2019 [10], and with an ever-expanding knowledge base there are now 5 different groups of PH and huge advances in treatment options which have had a profound impact on patients’ lives.

1.3 Definition of Pulmonary Hypertension

The gold standard test for diagnosing PH is RHC. In health at rest the normal mPAP is approximately 14.0 ± 3.3 mmHg. The definition of PH was recently amended following the 6th World Symposium from an mPAP ≥ 25 to now requiring an mPAP >20 mmHg. However, this lower mPAP is not felt sufficient alone to define PH and therefore the PVR; the measure of resistance against blood flow from the pulmonary artery to

the left atrium and the pulmonary artery wedge pressure (PAWP; an indirect estimate of the left arterial pressure) are now included.

The PVR is calculated as follows:

$$PVR = mPAP - mLAP / CO$$

(mLAP = mean left atrial pressure)

Using all three of these measurements in combination allows for differentiation of pre-capillary, post-capillary or combined PH. Table 2 details the values for diagnosis. Defining PH as pre- and post-capillary helps to classify the type of PH present.

1.4 Classification of Pulmonary Hypertension

PH is currently classified into 5 different diagnostic groups based on their pulmonary vascular pathophysiological mechanisms and clinical characteristics. (Table 1) Each diagnostic group is treated and managed differently, therefore recognising the main cause of a patient's PH is important.

In the United Kingdom (UK) there is a reported estimated total prevalence of 97 cases per million of all types of PH with a female: male ratio of 1.8. [11]

Table *1* **Classification of PH**

1. Pulmonary arterial hypertension
1.1 Idiopathic PAH
1.2 Heritable PAH
1.3 Drugs – and toxin – induced PAH
1.4 PAH associated with :
1.4.1 Connective tissue disease
1.4.2 Human Immunodeficiency Virus (HIV)
1.4.3 Portal hypertension
1.4.4 Congenital heart disease
1.4.5 Schistosomiasis
1.5 PAH long- term responders to calcium channel blockers
1.6 PAH with overt features of venous/capillaries (pulmonary veno-occlusive disease/ pulmonary capillary haemangiomatosis) involvement
1.7 Persistent PH of the newborn syndrome
2. Pulmonary hypertension due to left heart disease
2.1 PH due to heart failure with preserved left ventricular ejection fraction (LVEF)
2.2 PH due to heart failure with reduced LVEF
2.3 Valvular heart disease
2.4 Congenital/acquired cardiovascular conditions leading to postcapillary PH
3. PH due to lung disease and/or hypoxia
3.1 Obstructive lung disease
3.2 Restrictive lung disease
3.3 Other lung disease with mixed restrictive/obstructive pattern
3.4 Hypoxia without lung disease
3.5 Development lung disorders
4. PH due to pulmonary artery obstructions
4.1 Chronic thromboembolic PH
4.2 Other pulmonary artery obstructions
5. PH with unclear and/or multifactorial mechanisms
5.1 Haematological disorders
5.2 Systemic and metabolic disorders
5.3 Others
5.4 Complex congenital heart disease

A comprehensive list of disorders in each of the 5 groups of pulmonary hypertension. [12]

1.4.1 Group 1: Pulmonary Arterial Hypertension

PAH primarily affects the pulmonary vasculature. A destructive pulmonary arteriopathy occurs by progressive remodelling of the small pulmonary arteries, leading to vasoconstriction in the pulmonary arteries and arterioles, a progressive increase in PVR and progressing into right heart failure and ultimately death. [13] These

processes can be driven by several different factors including several genetic mutations, certain drugs and toxins, and associated conditions such as connective tissue disorders.

The change in pulmonary vasculature leads to an increase in PVR in PAH. This in turn leads to increased vascular load on the right ventricle (RV). The usually thin-walled RV adapts by increasing contractility (up to a 5-fold increase) to match this increased load, and in time hypertrophies. This increased contractility is achieved without an increase in stroke volume, and this is termed ‘coupling’. This adaptation initially allows the heart to cope with mild increases in pulmonary artery pressures, however as the disease progresses and pressures continue to rise, the ability of the RV to maintain sufficient contractility diminishes despite ventricular dilatation. RV failure ensues with ‘uncoupling’ of contractility against load, and the increasing metabolic demand caused by the raised heart rate to meet CO cannot be sustained. This then leads to reduced CO and failing of the RV. [14]

PAH has an estimated prevalence of 15 - 60 cases per million population and an incidence of 5 – 10 cases per million per year. Around half of these cases are patients who have idiopathic, heritable or drugs – and – toxin induced PAH. [11] The other half is largely made up of PAH in association with connective tissue disease followed by congenital heart disease and other rarer causes.

The age at which patients are diagnosed with PAH has increased. The first National Institute of Health registry created in 1981 found an average age of diagnosis of 36 years with a significant female predominance. PAH is now more commonly diagnosed in patients between the ages of 50-65 years and the previous female predominance is not so clear on data from more recent registries. [11]

1.4.1.1 Idiopathic, heritable and drugs or toxin induced PAH

Drug induced and heritable PAH (HPAH) is grouped with idiopathic PAH (IPAH) as clinically there are minimal differences between the groups, apart from there is evidence to suggest HPAH may present with less severe exercise impairment. [15, 16] IPAH and HPAH are rare in the general population and account for around 5 -15 cases per one million adults. [17] However, patients with sporadic PAH have been shown to have a high prevalence of genetic mutation and therefore HPAH may be underdiagnosed. [18] The term HPAH encompasses sporadic IPAH with genetic mutations and familial cases with or without known genetic mutations. [19]

Since 2000 there have been several advancements in knowledge regarding the genetics of PAH. Based on current knowledge, around 25-30% patients diagnosed with IPAH have an underlying genetic cause [20]. Mutation in the bone morphogenic protein receptor type ll (BMPR2) gene is thought to be the most common that predisposes patients to HPAH. This is an autosomal dominant disease, with incomplete penetrance. It is thought that the penetrance for BMPR2 gene is around 10% for men and 40% for women. Interestingly, 9 – 22.5% of patients who have PAH in association with anorexigens carry the BMPR2 gene. There have been several other PAH –predisposing genes identified such as activin A receptor ll-like kinase 1 (AKL1) and endoglin (ENG). These are more associated with the development of hereditary haemorrhagic telangiectasia (HHT), a condition which itself is associated with the development of PAH, but also rarely have been seen in isolation in the development of HPAH on its own. All of these gene mutations are involved in the transforming growth factor (TGF) –β signalling pathway which plays an important role in pulmonary vascular remodelling. [21]

Due to the nature of these mutations having incomplete penetrance, genetic testing is complex. However, in the UK testing can be performed on all consenting patients diagnosed with PAH. Genetic education and counselling should be performed prior to genetic testing. [20] Family members can also be screened if a mutation is identified. Asymptomatic family members who are found to be at risk should receive regular check-ups and be educated on symptoms to be aware of. [22]

PAH has been also associated with several drugs and toxins, most notably anorexigens. This has led to several upsurges in cases in the past, in particular in the 1960's due to aminorex, and in the 1990's due to fenfluramine. There are a number of other drugs and toxins that are associated with developing PAH. Many of the drugs have similar molecular similarities to amphetamines. [23]

1.4.1.2 PAH associated with connective tissue disease

Connective tissue diseases (CTD) are a varied collection of disorders that cause inflammation, vascular and fibrotic manifestations in multiple organs including kidneys, skin, and lung. [24] Systemic sclerosis (SSc) is the most common CTD associated with PAH. [25] There is a 10% prevalence of PAH in the SSc population and due to this high prevalence, there are screening programmes in place which use tools such as the DETECT algorithm to aid early diagnosis of these patients. Prognosis is poor, however early initiation of targeted PH treatment is associated with better survival outcomes. [26]

1.4.1.3 PAH associated with Human Immunodeficiency Virus

Patients with human immunodeficiency virus (HIV) have a higher incidence of PAH then the general population, it is thought approximately 1 in 200 patients with HIV will develop PAH. [27] Due to advancements in antiretroviral treatment for HIV with significantly improved survival, associated diseases such as PAH have now become a primary cause of morbidity and mortality. [28] Although screening for PAH in patients with HIV is not routine in the UK, it should be considered in the context of progressive dyspnoea in patients with HIV. [29]

1.4.1.4 Porto-pulmonary hypertension

Porto-pulmonary PH causes significant morbidity and mortality in patients with portal hypertension with or without liver disease, it is thought to be present in 2-10% of patients with portal hypertension. [30, 31] Although the haemodynamic profile of patients with porto-pulmonary is better than in patients with IPAH or HPAH (porto-pulmonary hypertension patients tend to exhibit higher CO) and lower PVR than in PAH), survival remains poor in comparison, 67 vs.85% at 2 years, and 40 vs, 64% at 5 years. [32] Furthermore, severe porto-pulmonary hypertension is associated with increased perioperative and post-operative risk for those patients undergoing liver transplant. Therefore, optimisation of porto-pulmonary hypertension is essential prior to transplant listing. [33]

1.4.1.5 PAH associated with congenital heart disease

PAH can develop in a number of congenital heart defects (CHD), however defects in the ventricular or atrial septum are most common. The prevalence of PAH in the presence of these septal defects is 6% [34], with half of these patients meeting the definition of Eisenmenger's syndrome [35], which is defined as a CHD with initially large systemic-to-pulmonary shunt, which in turn induces severe pulmonary vascular disease and PAH, resulting in reversal of the shunt direction and central cyanosis. [35] Without treatment the 3-year survival for patients with Eisenmenger's syndrome is 77% in comparison to patients with IPAH of 35%. [36] This is despite similar elevations in mPAP, due to the "training effect" of persistent and longstanding right ventricular hypertension, right atrial pressure (RAP) and CO remain better preserved in congenital PAH then IPAH.

1.4.2 Group 2: Pulmonary Hypertension due to left heart disease

PH due to left heart disease (PH-LHD) is the most common type of PH. The prevalence of PH in left heart failure varied significantly with diagnostic criteria from 25-83%. [37–40] It includes PH due to systolic or diastolic left ventricular dysfunction, mitral or aortic valve disease and congenital left heart disease. It is caused by back pressure across the pulmonary vascular bed, leading to an increase in pulmonary artery pressure to maintain an adequate driving pressure. Unlike IPAH, targeted PH medications have not shown benefit, and some have been related to increased mortality. Therefore, the mainstay of management is to optimise patients left heart failure. [41–43]

1.4.3 Group 3: Pulmonary Hypertension due to lung disease and/or hypoxia

There are multiple lung diseases that can lead to PH. Chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) are both common diseases in the general population and thus due to their high prevalence are the most common causes of PH. [44–46] However, other rarer lung diseases such as cystic fibrosis, Langerhans histocytosis and lymphangioleiomyomatosis (LAM) can also be associated with the development of PH. Hypoventilatory conditions such as obesity hypoventilation syndrome (OHS) are also common causes of PH. [47] The main goal of treatment is to optimise the underlying disease, for example using bronchodilators in COPD, or anti-fibrotic agents in IPF and long-term use of non-invasive ventilation (NIV) in OHS. Targeted PH medications in multiple studies have previously shown no benefit to patients and in some circumstances worsen outcomes. [19, 47, 48] However, more recent studies examining PH associated with IPF specifically have shown morbidity and mortality benefits from targeted PH treatments (discussed further in section1.7). [49–51] Patients with advanced lung disease and who are eligible should be considered for lung transplant. [48]

1.4.4 Group 4: PH due to pulmonary artery obstructions

Group 4 PH is mainly due to the development of CTEPH. This complication of PE is caused by chronic, organised clot in the pulmonary vasculature, leading to PH. [52] This topic will be discussed in more detail in section 1.15 as part of the focus of this M.D is on PE and the development of CTEPH.

Other forms of pulmonary artery obstructions are much rarer and include examples such as spindle cell sarcoma and Takayasu arteritis. These patients may present in a similar fashion to those with CTEPH however, it is important to differentiate as the

management will be dictated by their underlying aetiology and may include chemotherapy or immunosuppression rather than targeted PH therapies or surgical options. [53, 54]

1.4.5 Group 5: PH with unclear and/or multifactorial mechanisms

Group 5 PH is a heterogeneous group of diseases that are associated with PH secondary to multifactorial mechanisms. Causes include chronic myeloproliferative disease, sickle cell disease and thalassemia and also post-splenectomy. There is little literature surrounding these causes and no clear treatment guidelines. [55]

One group that is more well recognised is sarcoid associated PH. Sarcoid is a multisystem disease of unknown aetiology characterised by tissue infiltration with non-caseating granulomata. [56] The incidence of sarcoid and PH is estimated to be between 5-28% but could be as high has 75% in those awaiting lung transplant. [57, 58] Symptoms are often non-specific and overlaps with the symptoms of patients' lung disease and therefore PH often is underdiagnosed in this groups of patients. The cause of PH in the setting of sarcoid is multifactorial, it can result from left heart dysfunction due to myocardial involvement or the result of disruptions of the pulmonary vasculature caused by pulmonary fibrosis. However, PH can also be present in the absence of lung involvement and is likely due to granulomatous infiltration of the pulmonary arterioles resulting in a vasculopathy or due to extrinsic pressure on the pulmonary arteries by enlarged mediastinal lymph nodes. [59] Although there are limited data, patients with sarcoid- related PH in the UK do have access to targeted PH therapies, as small case series have shown some benefit. [60]

Table 2 **Summary of haemodynamic definitions of PH and corresponding clinical groups**

	Haemodynamic	Clinical groups
Pre-Capillary PH	mPAP >20 mmHg PAWP ≤ 15 mmHg PVR >2 WU	1, 3, 4 and 5
Post-Capillary PH	mPAP > 20 mmHg PAWP > 15 mmHg PVR ≤ 2 WU	2 and 5
Combined pre- and post-capillary PH	mPAP > 20 mmHg PAWP > 15 mmHg PVR >2 WU	2 and 5

Diagnostic haemodynamic parameters at RHC, defined by the ERS/ESC 2022 Guidelines for the diagnosis and treatment of pulmonary hypertension.[61]

Abbreviations: PH= pulmonary hypertension, mPAP= mean pulmonary artery pressure, PAWP= pulmonary artery wedge pressure, PVR= pulmonary vascular resistance

1.5 Existing Clinical Pathways for the Diagnosis of PH

Symptoms of PAH are non-specific and relate mainly to progressive RV dysfunction. Dyspnoea on exertion is often the first symptom, along with fatigue, weakness, angina and syncope. Symptoms at rest only occur in more advanced cases, and as RV failure ensues patients may develop peripheral oedema and pericardial effusions. [11] As these symptoms are non-specific they commonly and understandably can be mistaken for more common pathologies, which is why there is a well-recognised delay in presentation to diagnosis of PAH of normally around 4 years from the patient first reporting symptoms to a healthcare professional to diagnosis. [62]

Once suspicions of PAH are raised patients are referred to a specialist adult pulmonary vascular disease centre. In the UK these are based in Newcastle, Glasgow, London, Cambridge and Sheffield.

1.6 Investigations

Following history and examination, patients where PH is suspected will go on to have a range of investigations (if appropriate) to aid formal diagnosis to allow for categorisation of type of PH and severity of the disease.

Investigations include blood tests, that along with routine bloods include more specific to PH diagnosis HIV serology, thrombophilia testing and an immunology screen, particularly examining for CTD that have associations with PH such as limited cutaneous systemic sclerosis are performed. [10] NT-proBNP is also measured and is important in the risk stratification of patients who go on to be diagnosed with PH, this will be discussed in more detail in section 1.10.

Chest x-ray can demonstrate features of PH such as cardiomegaly, central pulmonary artery dilation and pleural effusions (which could be related to PH-LHD, sarcoid or pulmonary veno-occlusive disease (PVOD)), [63] and may also demonstrate if any lung parenchymal disease present.

Pulmonary function tests such as spirometry can offers information regarding the presence of any underlying lung disease. The lung diffusing capacity of carbon monoxide or transfer factor (TLco). TLco can be low in IPAH [64] and is often low in CTEPH. [65] TLco has been proven to be a prognostic marker in PAH. [66]. Also, overnight pulse oximetry maybe a useful tool to investigate for the presence of

obstructive sleep apnoea (OSA) or OHS which can be a contributing factor worsening PH.

Although, these tests may be abnormal in a large proportion of patients with PH, they are non-specific and most do not correlate with disease severity, however help to build the clinical picture of the patient. [67, 68] In 80-90% of patients with PH their chest x-ray and/or ECG are abnormal at diagnosis. [69]

More detailed and specific investigations for the presence, type and severity of PH are performed such as functional assessments, imaging which includes transthoracic echocardiogram (ECHO), cardiac magnetic resonance imaging (MRI) and CT. The gold standard investigation to confirm the diagnosis of PH is by performing a RHC which gives an accurate picture of the pulmonary haemodynamic, parameters which are used to formally diagnose PH.

1.6.1 Walking Test to assess exercise capacity

As it is not practical or appropriate to repeat invasive tests such as RHC or expensive imaging such as CMR every time a patient is reviewed in clinic, other easier to perform and non-invasive tests are used to assess patients' clinical status. Exercise tests, although they have an element of effort-dependence, are a useful tool. In PH, either the 6-minute walking test (6MWT) or incremental shuttle walking test (ISWT) can be performed. The 6MWT is a simple test that requires no exercise equipment, the test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes. The self-paced 6MWT assesses the submaximal level of functional capacity; patients are allowed to stop and rest during the test. [70] As most activities of daily living are performed at submaximal levels of exertion, the 6MWT may better

reflect the functional exercise level for daily physical activities [71] and it has been shown to correlate with haemodynamic parameters in IPAH and predict survival at baseline and follow up. [66, 72] However, the 6MWT is thought to correlate loosely with aerobic capacity [73] and there are concerns about a “ceiling effect” that may mask efficacy in patients with less severe symptomatic disease who have high baseline walk distances but, nevertheless, may have substantial pathology. [74] Despite this the 6MWT is more widely used in PH centres across the world. Less commonly used, but the method preferred in Sheffield Pulmonary Vascular Disease (SPVDU) unit is the ISWT, where patients are asked to walk at 10-m in length keeping in time to an external audible signal. Level one consists of three lengths (30 m), and each subsequent level adds one extra length to the preceding level. The initial speed is a slow walk, incrementally increasing at every level. Each level takes 1 minute to complete and finishes at the end of level 12 or until the patient is too breathless or unable to keep up with the required pace. In comparison to the 6MWT this is a maximal test and has been shown in PAH to stratify mortality risk at baseline and follow-up. [75]

1.6.2 Echocardiogram

Transthoracic echocardiogram (TTE) is a useful non-invasive method of providing details of cardiac structure and function, although not as accurate as CMR and RHC, it is relatively inexpensive and does not carry the same risks as RHC. It is usually recommended as a first line assessment of cardiac function if PH is suspected and has good sensitivity (0.79–1.0) and specificity (0.60–0.98) but can also under- and over-estimate systolic pulmonary arterial pressure (sPAP). [76] Features suggestive of PH on TTE along with a raised sPAP include dilated right sided chambers, reduced RV function, RV hypertrophy, enlarged pulmonary artery and abnormal interventricular

septal motion. However, while TTE is a useful initial tool for the evaluations of cardiac chamber size and function and has actually been incorporated into early detection of PH programmes for patients with SSc and has shown to be a reliable tool in this setting [77, 78], it is important to recognise the limitations of TTE. It may frequently be inaccurate in estimating pulmonary artery pressures and CO in patients being evaluated for PH. [79]

1.6.3 Magnetic resonance imaging

CMR is increasingly used in the non-invasive monitoring of patients with PH. Comparing MRI to TTE, it provides a more accurate picture of cardiac function, lower operator dependence and greater interstudy reproducibility. [80] A systematic review, including 1,938 patients showed that CMR is a powerful predictor of clinical worsening and mortality. [81] In particular, worse RV function and larger RV volume were associated with worse outcomes. A further study of 576 patients demonstrated that RV end-systolic volume and pulmonary artery (PA) relative area change had incremental prognostic value over other clinical parameters. [82] A low relative change of the PA indicated increased PA stiffness which was associated with more severe PH and a higher risk of mortality. [82–84]

CMR has been used in validated prognostic calculators such as the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) and demonstrated improved prognostication when combined with risk scores particular in age- and sex- adjusted RV end-systolic volume index. [85]

1.6.4 Computerised tomography scan

CT is a valuable imaging modality for the evaluation of known or suspected PH. CT has the advantage of being readily available in most hospitals and has the ability to evaluate pulmonary vasculature, lung parenchyma, cardiac, and mediastinal structures. [86].

In the presence of PH, the pulmonary vasculature may show an enlargement of the main PA greater than the accompanying aorta or ≥2.9 measured at the level of the pulmonary artery bifurcation. [87] CT angiography is the method of choice for the evaluation of the pulmonary vasculature in the context of suspected CTEPH, where multiple findings can be seen such as intravascular organising thrombi, webs and vascular narrowing or occlusion. [88] Mosaicism of the lung parenchyma and enlarged bronchial arteries may also be a feature. [89, 90]

CT also provides a detailed assessment of the lung parenchyma. Centrilobular ground-glass opacities are frequently seen in PAH and in the context of unexplained breathlessness should highlight the possibility of PAH. [91] In a large registry series, other features such as cardiac decompensation, pleural effusion/septal lines and inferior vena cava size have been shown to predict outcome. [91, 92] The presence of lung disease such as emphysema, interstitial lung disease or bronchiectasis make the diagnosis of PH in association with lung disease more likely. CT may also be helpful in the identification of rarer conditions such as PVOD and pulmonary capillary hemangiomatosis (PCH). [93]

Although CT is not the gold standard method of evaluating cardiac abnormalities, many cardiac findings associated with PH can be identified with non-gated contrast-enhanced CT. These findings include cardiac chamber enlargement, thickening of the

RV free wall, and left deviation of the interventricular septum and abnormalities associated with CHD. [93]

In addition to the above, CT can also provide clues from the mediastinal anatomy, such as a dilated oesophagus which may suggest a diagnosis of SSc and other findings such as pericardial effusions, lymphadenopathy and reflux of contrast into the hepatic veins which although not specific, if present in the context of PH may suggest a poorer prognosis. [94]

1.6.5 Isotope Perfusion lung scan

The European Society of Cardiology / European Respiratory Society (ESC/ERS) guidelines suggest perfusion lung scans (V/Q scintigraphy) scans should be performed in all patients with unexplained PH, to assess for the presence of CTEPH. [11] Although there has been increasing use of computed tomography pulmonary angiography (CTPA) in the diagnosis of CTEPH, V/Q scans have a greater sensitivity. A study examining 227 patients, found V/Q scintigraphy had a sensitivity of 96-97% and a specificity of 90-95% in comparison to CTPA which showed a sensitivity of 51% and a specificity of 99%. [95] Although interpretation can be difficult, a normal V/Q scan excludes the presence of CTEPH. Most patients with CTEPH have very abnormal V/Q scans findings and often have multiple moderate and large perfusion defects, without matching ventilation defects, in their lungs. [95, 96] A large defect generally involves more than 75% of the expected volume of a bronchopulmonary segment, a moderate defect 25-75% and small defect less than 25% of the expected segmental volume. [97]

1.6.6 Right Heart Catheterisation

RHC is the gold standard investigation for diagnosis PH, disease severity and tests pulmonary vasoreactivity. It is an invasive procedure that allows direct measurement of the right-sided cardiac pressures and calculation of CO. It allows for accurate measurements of the RAP, PAWP, mPAP, mixed venous oxygen saturations, CO, cardiac index (CI) by thermodilution. [98] Vasodilator testing can also be performed during the RHC, this is performed by the administration of either epoprostenol or nitric oxide. A response occurs in <10 % of patients. Acute responders are defined by a fall in mPAP of ≥ 10 mmHg to reach an absolute values of ≤ 40 mmHg with an increased or unchanged CO. These patients who are responders should be trialled with high-dose calcium channel blockade. [99] Usually around 50% of acute responders continue to have long-term response to calcium channel blockers, these patients have excellent long-term haemodynamic improvement and prognosis, with the average seven-year survival of 97%. [100, 101]

RHC is considered a safe procedure, when performed at specialist pulmonary vascular disease centres. A large study of 7,218 RHC, found the morbidity and mortality rates to be low, (~1% and <0.1% respectively), with the most frequent complications relating to venous access (haematoma and pneumothorax), followed by arrhythmias and hypotensive episodes. [102]

1.7 Treatment options

In the setting of group 1, over the past two decades there have been significant improvements in patient outcomes. [103] Treatment of PAH primarily encompasses pharmacological and lifestyle interventions. Current pharmacological therapies target

three distinct biological pathways (prostanoid, endothelin and nitric oxide), with evidence for superiority of combination therapy over monotherapy. [11] The aim of therapy is to maintain or achieve a low-risk profile, thus establishing the severity of disease and serially assessing response to therapy is key to informed decision making.

Clinical trials studying group 2 PH (due to LHD) have not demonstrated a benefit in treating with targeted pulmonary hypertension therapies, in fact some have been related to increased mortality. [104–108] Therefore, optimising patients left heart disease is key in managing PH due to LHD.

In PH in lung disease (group 3), the role of pulmonary vasodilators is contentious [109], in patients with COPD, sildenafil and bosentan have shown improvements in pulmonary haemodynamics, but this has not been seen in exercise capacity or quality of life. [110–112] In the setting of interstitial lung disease (ILD), randomised controlled trials and meta-analysis previously have shown varied and inconsistent results for the use of targeted PH treatments in this cohort. Even thoughts that pulmonary vasodilators could worsen hypoxia by attenuating ventilation/perfusion mismatching in areas of fibrosis. [111, 113, 114] However, recent studies have provided some hope for treatment options for patients with ILD-PH. A retrospective, observational study of 128 ILD-PH patients in the UK reported an increase in median survival of 2.18 years with phosphodiesterase 5 inhibitors (PD5i) treatment compared to 0.94 years in untreated patients. The survival difference was larger (2.55 years) in patients with normal RV function at initiation of PDE5i. [49] The INCREASE trial has demonstrated reduction of NT-proBNP levels and increased exercise capacity in patient with ILD-PH who used inhaled Treprostinil. [50, 51]

Group 4 PH (CTEPH) like PAH has seen a huge advancement in treatment options and therefore increased survival. [113] Along with targeted PH medications, surgical interventions have made CTEPH now a potential curable condition. [52]

Group 5 PH consists of a wide varying complex group of disorders. Studies investigating treatments in this group are limited and involve small numbers of patients. Therefore, treatments should be individually tailored to each patient, with the underlying condition optimised as much as possible in the first instance. [114]

1.7.1 Targeted Pulmonary Hypertension Therapies

1.7.1.1 Calcium Channel Blockers

The use of calcium channel blocker in PAH is only reserved for those patients who show a significant immediate haemodynamic response to pulmonary vasodilators (either epoprostenol or nitric oxide) at the time of RHC. It is suggested that around 5-10% of patients with IPAH are ‘vasoresponders’. [115, 116] However, those patients that do respond tend to have a sustained long-term response, with studies demonstrating significantly low mPAP and PVR and with the majority of patients after 7 years of follow-up remaining in (New York Heart Association Functional Class) NYHA FC class I or II. [99]

1.7.1.2 Phosphodiesterase-5-inhibitors

Drugs such as sildenafil and tadalafil inhibit PDE5 i, an enzyme that metabolises cyclic guanosine monophosphate (GMP), which is involved in controlling the relaxation and growth inhibition of vascular smooth-muscle cells, including those in the lung.

Treatment with such drugs have been shown to improve exercise capacity, (World Health Organisation functional class) WHO FC and pulmonary haemodynamics in patients with PAH. [117–119] PDE5 i if tolerated tend to be 1st line or used in combination with other targeted PH therapies. There is clinical evidence of improvement in patients with IPAH, PAH-CTD, PAH-CHD, CTEPH and more recently PH in the context of ILD when treated with PDE5 i. [120–122]

1.7.1.3 Endothelin-1 receptor antagonists

Endothelin is a potent vasoconstrictor and smooth-muscle mitogen and plays an important role in the pathogenesis of PAH. Endothelin-1 receptor antagonists (ERA), such as ambrisentan, bosentan and macitentan, are all currently available for the treatment of PH. [123]They work by binding to receptors of endothelin and have shown to improve prognosis of PAH by delaying the progression of disease. [124, 125] The ERA's have also been shown to be effective in inoperable CTEPH [126] and also patients with PAH-CHD who following treatment had an improved exercise capacity and haemodynamics without compromising peripheral oxygenation saturations. [127]

1.7.1.4 Soluble guanylate cyclase stimulators

Riociguat, which increases the production of cyclic GMP. This has been approved for the treatment of patients with PAH and also patients with CTEPH that is persistent/recurrent or non-operable. [128, 129] The PATENT-1 study showed significant improvements in 6MWD, PVR, NT-proBNP and WHO FC compared to placebo. [130] It can be used as a monotherapy or in combination with an ERA.

1.7.1.5 Selective prostacyclin receptor agonist

Selexipag is an oral selective prostacyclin receptor agonist. The dose is titrated over several weeks to a maximum dose tolerated. Clinical trials showed a 30% reduction in PVR after 17 weeks of treatment. [131] It is approved from patients with PAH who are in WHO FC II/III. It can be used in combination with an ERA or PDE5 i in patients insufficiently controlled with either agent or as a monotherapy in patient who have not tolerated a PDE5 i or ERA. [132]

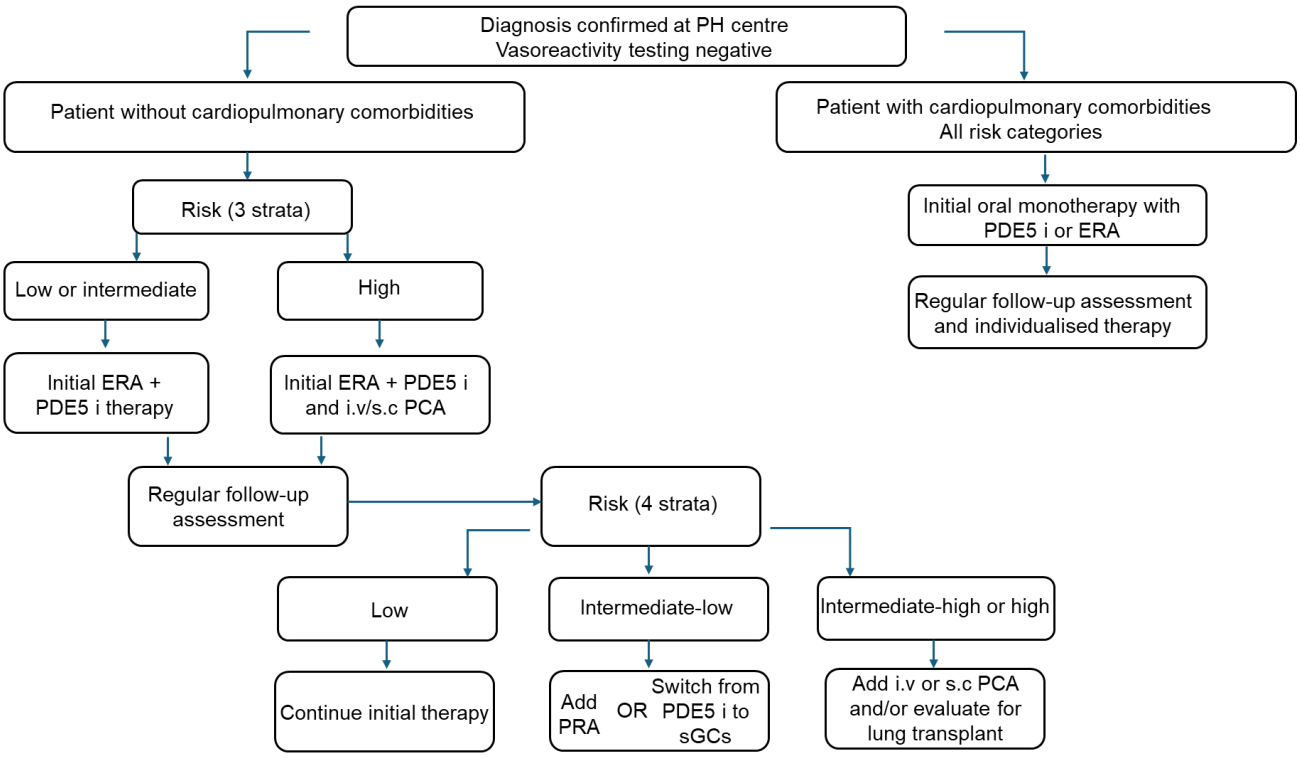
1.7.1.6 Prostanoids

Prostanoids are analogues of prostacyclin and can be administered by several different routes including oral, continuous intravenous infusion, continuous subcutaneous infusion or inhaled as a nebuliser. [133] These drugs are potent vasodilators and have antiproliferative and anti-inflammatory properties. [134] Prostanoids in their different arrangements have been shown to be effective in most forms of PAH and CTEPH. [135–139] One of the main considerations of these treatments is the technical ability needed for patients to be able to be independent with route of administration of the prostanoids, most routes require digital dexterity and a significant amount of training, this is some circumstances can be the limiting factor for patients being suitable for prostanoids.

1.7.2 Selection of medical management

Guidance for medical management of patients with PAH is based upon a patient's risk stratification (discussed further in section 1.9). Following the assessment of several clinical parameters patients fall in to either low, intermediate or high-risk groups which correlate with increasing 1-year mortality. [140] The aim of medical management is to reduce patients' risk to the low-risk category, therefore at regular intervals risk is reassessed and if appropriate addition layers of medical management at added (Figure 1).

Figure 1 **Risk stratification and medical therapy of PAH**



PAH treatment algorithm for patients with idiopathic, heritable, drug-associated, and connective tissue disease-associated PAH. Adapted from Galie et al. [61] (CC BY-NC 4.0).

Abbreviations: ERA= endothelin receptor antagonist, i.v= intravenous, PDE5 i= phosphodiesterase 5 inhibitor, P = pulmonary hypertension, PRA= prostacyclin receptor agonist, s.c= subcutaneous, sGCs= soluble guanylate cyclase stimulator

1.8 Surgical Management

1.8.1 Pulmonary Endarterectomy

PEA provides a potential cure for patients with CTEPH. It is a complex surgical procedure which involves a midline sternotomy, patients put on cardiopulmonary bypass and made hypothermic to allow circulatory arrest. This allows the surgeon to work in a bloodless field to remove the occluding material in the pulmonary arteries. [141, 142] To be considered operable a patient must have surgically accessible

thromboembolic disease, with a proportional PVR indicating the absence of extensive distal disease. [143] Thrombotic disease located proximally in the main, lobar or segmental arteries can be removed relatively easily by PEA. However, distal disease in subsegmental vessels is more difficult to clear, making the patient inoperable. [52] Ultimately, individualised risk/benefit ratio for the patient is decided, determining the surgical final decision. The majority of patients have symptomatic and prognostic benefit even with those that remain with residual CTEPH. In a retrospective review of 142 patients 3 months post-PEA surgery 88% were WHO FC I or II in comparison to 12% pre-surgery, and pulmonary haemodynamic assessment demonstrated a 21mmHg fall in mPAP and a 105m increase in 6MWT at the 3-month mark post PEA. For patients who remained with persistent PH post PEA follow-up data demonstrated a persistent increased 3-year survival at 94% comparable to those patients post PEA without PH at 93%. [144]. This is in contrast to a 3-year survival of just 10% in an era prior to targeted PH treatments and PEA surgery. [145] In experienced centres mortality rates are <5%. Complications include reperfusion lung injury, neurological complications and persistent PH. [142] In the UK, all pulmonary endarterectomies are performed by a single centre at the Royal Papworth Hospital in Cambridge. [146]

1.8.2 Balloon pulmonary angioplasty

Since 2015, the Royal Papworth Hospital has been offering balloon pulmonary angiogram (BPA), which is a surgical intervention that can be offered to patients who are inoperable due to co-morbidities or other factors but have anatomically suitable disease. It has been shown to be safe and improves functional status, pulmonary haemodynamics and RV dimensions. Balloon mounted catheters are introduced over a guide wire into pulmonary segmental and subsegmental diseased vessels and inflated to unblock and restore flow. [148] Meta-analysis suggests BPA maybe superior

to medical therapy and possibility equivalent to PEA [149], although no randomised control trial comparing the different treatment options have been reported to date. Patients often have to attend several sessions over a period of time (three to five being typical) and the interval between sessions varies from 3 days to 1 month or more. Individual procedures are limited by the volume of lung treated, usually targeting one or two lobes per session. [150, 151] Complications rate is approximately 10% with common complications being haemoptysis, insertion site haematomas and lung reperfusion injury, mortality is generally reported as being <2%. [147]

1.8.3 Transplantation

Despite many advances in medical treatments and surgical interventions, some patients continue to deteriorate. Therefore, lung transplantation maybe an option for some patients. Due to the unpredictable course of PH, patients should be referred for lung transplantation at an early stage. [152, 153] They require formal assessment for their suitability for surgery, early referrals allow time to address any modifiable risk factors. Contraindications for lung transplant may include failure of other organs, obesity, malignancy and history of non-adherence to medical therapies. [152, 154] Most patients with PH receive bilateral lung transplants [155], although in some situations such as CHD, bilateral lung and heart transplant maybe required. [156] Survival rates post-transplant are improving over time. For all indications between 1990-1997 survival post-transplant was 4.3 years, by 2005 -2012 this had increased to 6.3 years. [157]

1.9 National Audit of Pulmonary Hypertension

In the UK there are 7 centres that are designated to diagnose and treat PH in adults and 1 centre designated for children. All centres participate in a national audit process annually, the latest published audit to date was the 14th audit, December 2023. The annual audit aims to answer 3 questions – Are patients receiving the right treatments in a timely manner? Are pulmonary hypertension services appropriate? What are the outcomes for patients with pulmonary hypertension? The audit uses a number of professionally agreed National Standards to measure clinic practice as well as analysis on referrals, treatments and survival to examine the quality of care provided to people referred to the PH services to answer these questions. [158]

1.10 Risk Stratification in PAH

Having reviewed the aetiology and classification of PH and the treatment of PAH, the next section will focus on how patients are stratified by risk of disease progression and mortality.

Risk stratification tools in medicine are common, but they are important. It is becoming recognised that PAH patients are a heterogeneous group and having the ability to risk stratify an individual and highlight to the clinician those patients that are more likely to rapidly decline can prompt escalation of treatments.

Current methods of risk stratification of patients with PAH are based around the assessment of symptoms, exercise capacity and RV function. There are a number of risk stratification scores including REVEAL 2.0 [159], Comparative, Prospective

Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA) [160] and the European registries based on the ESC/ERS. [11]

Several studies have supported the ESC/ERS guidelines when risk stratifying patients and found those deemed low risk have a longer survival than those in the high-risk groups. A large cohort of patients with IPAH (n=1017) from the French Pulmonary Hypertension Registry found patients achieving all three low-risk criteria of WHO/ NYHA FC I or II, 6MWD >440 m, BNP <50 ng·L−1 or NT-proBNP <300 ng·L−1) had 2-, 3- and 5-year survival of 100%, 99% and 97%, respectively. [161] This method has since been validated in data from the Phase 3 GRIPHON trial, which reported a 94% reduced risk of morbidity/mortality in patients with all three low-risk criteria versus those with no low-risk values. [162]

In contrast to the ESC/ERS guidelines, REVEAL assigns an overall score based on multiple demographic, haemodynamic and serum parameters in order to establish risk of 1-year mortality. Table 3 provides a comparison between ESC/ERS and REVEAL 2.0 risk stratification tools. An independent cohort examining REVEAL 2.0, has recently been published, demonstrated 1-year mortality estimates of 2.6%, 8.6% and 25.4% for low-, intermediate- and high-risk groups respectively, with 5-year estimates of 16.1%, 41.5% and 88.0%. [163]

Biomarkers BNP and NT-proBNP are integral to most risk stratification tools in PAH. The following sections will discuss these in more detail.

Table 3 **Comparison of ESC/ERS and REVEAL 2.0 prognostic tools**

	ESC/ERS guidelines			REVEAL 2.0 risk score calculator[#]		
Variable	**Low risk: <5%**	**Intermediate risk: 5–10%**	**High risk: >10%**	**−1 unless indicated**	**+1**	**+2 unless indicated**
WHO PH Group 1 subgroup					APAH-CTD	Heritable PoPH (+3)
Demographics						Male, age >60 years
Clinical signs of right heart failure	Absent	Absent	Present			
Comorbidities					eGFR<60 mL·min^{-1}/1.73 m^2 or renal insufficiency	
Symptom progression	No	Slow	Rapid			
Vital signs					SBP <110 mmHg HR >96 beats·min^{-1}	
Syncope	No	Occasional syncope	Repeated syncope			
All-cause hospitalisations ≤6 months					⩾1	
NYHA/WHO FC	I, II	III	IV	I	III	IV
6MWD	>440 m	165–440 m	<165 m	⩾440 m (−2) 320–<440 m	<165 m	
CPET	Peak V'_{O_2} >15 mL·min^{-1}·kg^{-1} (>65% pred) V'_E/V'_{CO_2} slope <36	Peak V'_{O_2} 11–15 mL·min^{-1}·kg^{-1} (35–65% pred) V'_E/V'_{CO_2} slope 36–44.9	Peak V'_{O_2} <11 mL·min^{-1}·kg^{-1} (<35% pred) V'_E/V'_{CO_2} slope ⩾44.9			
BNP/NT-proBNP plasma levels	BNP <50 ng·L^{-1} NT-proBNP <300 ng·L^{-1}	BNP 50–300 ng·L^{-1} NT-proBNP 300–1400 ng·L^{-1}	BNP >500 ng·L^{-1} NT-proBNP >1400 ng·L^{-1}	BNP<50 ng·L^{-1} NT-proBNP <300 ng·L^{-1}	BNP 200–800 ng·L^{-1}	BNP ⩾800 ng·L^{-1} NT-proBNP ⩾1100 ng·L^{-1}
Imaging/ echocardiography	RA area <18 cm^2 No pericardial effusion	RA area 18–26 cm^2 No or minimal pericardial effusion	RA area >26 cm^2 Pericardial effusion		Pericardial effusion	
Haemodynamics/ right heart catheterisation	RAP <8 mmHg CI ⩾2.5 L/min/m^2 S_{vO_2} >65%	RAP 8–14 mmHg CI 2.0–2.4 L/min/m^2 S_{vO_2} 60–65%	RAP >14 mmHg CI <2.0 L/min/m^2 S_{vO_2}2 <60%	PVR <5 Wood units	Mean RAP >20 mmHg within 1 year	
Pulmonary function test					D_{LCO} <40% pred	

Reproduced from Lewis et al [164] (CC BY-NC 4.0)

Abbreviations: ESC/ERS= European Society of Cardiology/ European Respiratory Society, REVEAL= Registry to evaluate early & long-term PAH disease management, WHO FC= world health organisation functional class, PH= pulmonary hypertension, e GFR= estimated glomerular filtration rate, SBP= systolic blood pressure, NYHA= New York heart association, RAP= right atrial pressure, BNP= brain natriuretic peptide, NT-proBNP = N-terminal prohormone of brain natriuretic peptide

1.11 The use of biomarkers in pulmonary arterial hypertension

1.11.1 Introduction to the uses of BNP and NT-proBNP

The active BNP and the functionally inert NT-proBNP are well-established clinical biomarkers used in PAH and other cardiovascular disorders such as acute and chronic heart failure and are used as substitute markers of cardiac function. [165] NT-proBNP is also used in the setting of SSc to screen for PAH in these patients. [26]

Typically, BNP or NT-proBNP are measured when patients are assessed at their specialist PAH centre at each appointment and these results are interpreted in conjunction with the results of other investigations. Venous blood samples are used for the clinical laboratory-based assays and it is usual that results are not immediately available while the patient is in attendance for their appointment.

1.11.2 Physiology of natriuretic peptides and the role of BNP and NT-proBNP

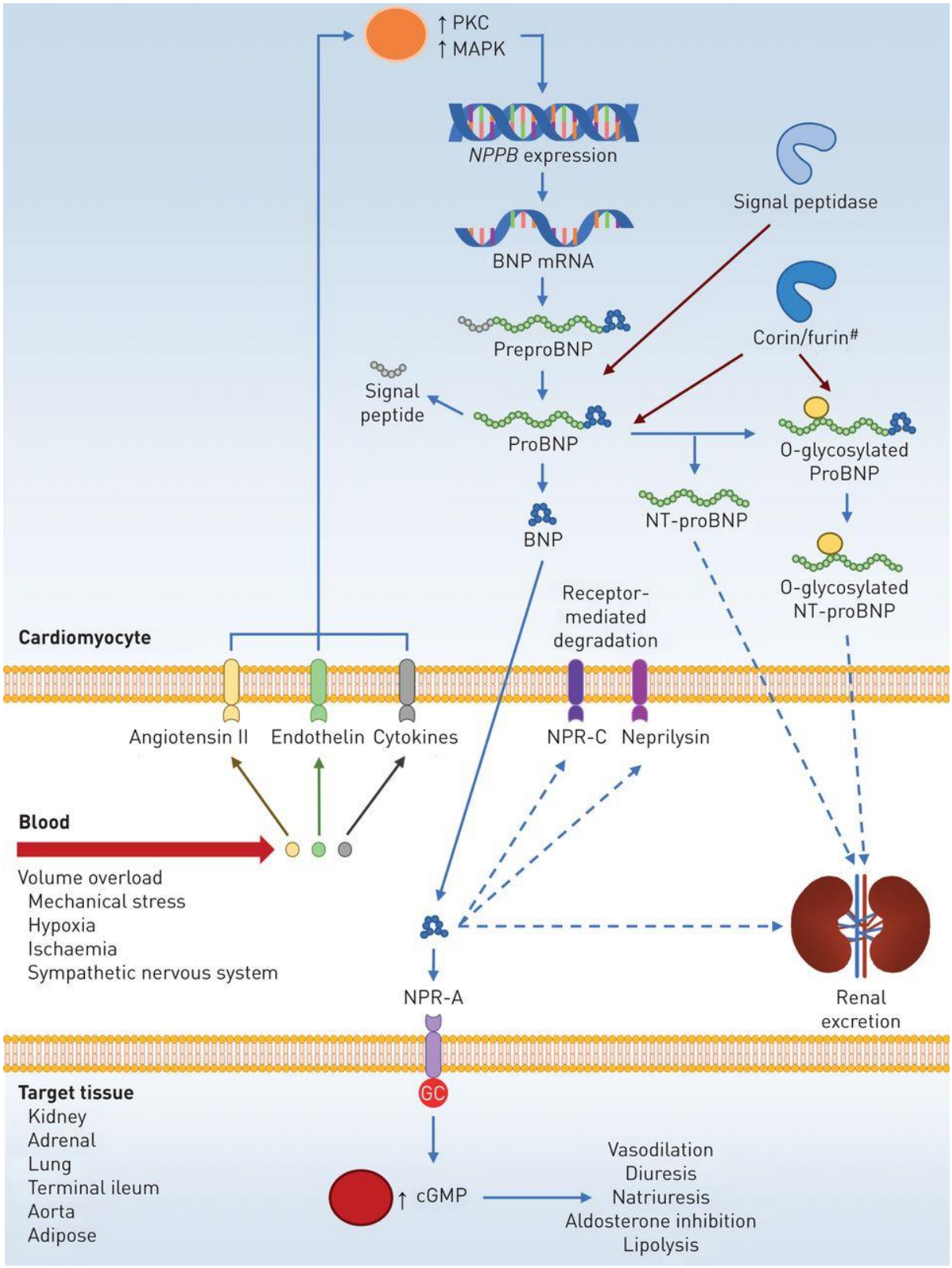
Natriuretic peptides are a group of hormones secreted primarily from the heart, kidneys and brain that cause vasodilation and natriuresis. They include atrial natriuretic peptide, BNP, C-type natriuretic peptide and urodilatin. Atrial natriuretic peptide has a short half-life of ~2 min and is sensitive to temperature change. In contrast, BNP has a longer half-life of ~22 min and is more stable, making it more amenable to use as a clinical biomarker. [166]

BNP is a product of the early-response gene natriuretic peptide B (*NPPB)*. It is mainly synthesised de novo and secreted by the ventricular myocardium in response to

mechanical, hormonal or sympathetic stimulation, with levels in blood peaking ~1 h after stimulation. [167]

In PAH, transmural pressure, volume overload, hypoxia or pro-inflammatory factors induce transcription of *NPPB* to produce 134-amino acid (aa) preproBNP. A signal peptide is subsequently removed in the sarcoplasmic reticulum, leaving 108-aa proBNP. This is then cleaved on secretion into the bloodstream to produce the two biomarkers of 32-aa BNP and 76-aa NT-proBNP (figure 2). [168] BNP binds to the natriuretic peptide receptor-A, which is primarily expressed in kidney, adrenal, lung, terminal ileum, aorta and adipose tissue. Receptor activation leads to increases in the intracellular secondary messenger cyclic guanosine monophosphate, resulting in vasodilation, natriuresis, aldosterone inhibition and lipolysis. [169] NT-proBNP has no known function but due to its longer half-life compared with BNP (70 min versus 22 min) and relative stability in storage, it has potential handling advantages as a biomarker. [170]

Figure 2 **Molecular pathways of synthesis and release of BNP and NT-proBNP**



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Abbreviations: aa= amino acid, cGMP= cyclic guanosine monophosphate, GC= guanylyl cyclase, MAPK= mitogen-activated protein kinase, NPR= natriuretic peptide receptor, PKC= protein kinase C

1.11.3 BNP and NT-proBNP correlation with pulmonary haemodynamics, echocardiographic and cardiac magnetic resonance imaging

BNP and NT-proBNP have been shown to correlate with several pulmonary haemodynamic metrics that are associated with survival. It has been shown that BNP correlates with RAP; r=0.66 at the time of RHC in patients with PAH. [171] It has also been demonstrated in SSc-PAH that NT-proBNP correlated with mPAP (r=0.62), PVR (r=0.81), RAP (r=0.53) and cardiac index (r= −0.50). NT-proBNP has been found to be an independent predictor of survival. [172] In a study of 30 patients undergoing RHC and CMR at baseline and follow-up, changes in NT-proBNP correlated with changes in RAP (r=0.49), CI (r=−0.45), PVR index (r−0.30), RV end-diastolic volume index (r=0.59), RV mass index (r=0.62) and right ventricular ejection fraction (RVEF; r=−0.81). [173]

In PAH correlations between BNP levels and TTE-measured sPAP have been shown, when left ventricular (LV) dysfunction was excluded (r=0.51). [76] A recent study focused on patients with PAH that were WHO FC III, where patients underwent blood testing for BNP, RHC and TTE, BNP levels and had positive correlations with RHC-measured RAP (r=0.20), mPAP (r=0.25) and PVR (r=0.31), and negative correlations with cardiac output (CO) (r=−0.33) and CI (r=−0.28). Using TTE, BNP positively correlated with right atrial (r=0.18) and RV enlargement (r=0.17), and RV dysfunction (r=0.15). BNP levels also correlated with 6MWD (r=−0.75) and higher WHO FC (r=0.26). Levels of BNP and NT-proBNP have also been shown to correlate with CMR measures of RV structure and function in small studies.

Similar correlations have been reported in PAH and CTEPH (n=25), with a significant association of NT-proBNP and CMR-RVEF (r=−0.66). One study also reported a sensitivity and specificity of NT-proBNP for detecting RV systolic dysfunction, defined as CMR-derived RVEF >2 SD below controls of 100% and 94%, respectively. [174]

1.11.4 BNP and NT-proBNP use in current practice

BNP and NT-proBNP are incorporated in several risk stratification tools for patients with PAH as mentioned previously, but interestingly as an extension to the ESC/ERC risk stratification guidelines it has also been identified that replacing RAP and CI with BNP/ NT-proBNP could also discriminate prognostic groups. [161] This is a particularly interesting point as this may have the potential in the future to reduce the requirement for expensive and invasive haemodynamic evaluation.

BNP and NT-proBNP are also used to screen for PAH in connective tissue disease, especially SSc. A study of 109 patients with SSc and NT-proBNP >395 $ng \cdot L^{-1}$ found a positive predictive value of 95.1% (sensitivity 55.9%/specificity 95.1%) and negative predictive value of 56.5% for association with PAH. Changes in NT-proBNP levels were demonstrated to be highly predictive of mortality with a five-fold increased risk of dying for every 10-fold increase in baseline NT-proBNP level (HR=4.82). [172] The DETECT study proposed a novel algorithm based on ESC/ERS guidelines for screening patients with SSc for PAH and this algorithm includes NT-proBNP. The Australian Scleroderma Interest Group (ASIG) has also recently incorporated NT-proBNP into a screening algorithm. [175]

1.11.5 BNP and NT-proBNP sampling considerations in clinical practice

1.11.5.1 Physiological variables to consider

Elevated BNP and NT-proBNP are not specific markers for PAH and can be seen in several clinical settings. They can be raised in not only PAH, but other cardiovascular pathologies including LV dysfunction and LV hypertrophy. [169] BNP and NT-proBNP results must be put into clinical context when interpreting, as values are not only increased by ventricular stress but also increased by several demographic factors such as increasing age, female sex and body weight, as well as the presence of chronic renal failure, type 2 diabetes mellitus, anaemia, PE and acute coronary syndrome. [176] In renal failure circulating levels of BNP and NT-proBNP inversely correlate with kidney function, however this effect is much more pronounced for NT-proBNP as it is excreted exclusively into urine. [177]

High intra-individual biological variation in patients with stable heart failure has been reported, with reported week-to-week reference change values of 49.2% and 66.2% for BNP and NT-proBNP respectively. [178] Additionally, it has been noted that there may be a circadian component with significant increases of BNP during the day, peaking at >120% of the 08:00 h baseline at 18:00 h. There is less evidence for significant daily NT-proBNP variation. [179]

Another important variable is the effect exercise may have on BNP and NT-proBNP levels. A systematic review of the effects of running demonstrated that 22.9% and 35.9% of individuals who had completed races of varying length and intensity had BNP and NT-proBNP levels above the upper reference limit (typically 100 $pg \cdot mL^{-1}$ for BNP, which is the recommended acute heart failure threshold [180], and 125 $pg \cdot mL^{-1}$ for

NT-proBNP) respectively, when measured <24 h after an event. [181] Rapid transient plasma BNP increases well below 100 $ng \cdot L^{-1}$ have also been observed in healthy individuals undertaking short-term maximal exercise, peaking at a mean 30.6±4.7 $ng \cdot L^{-1}$ from a resting level of 19.4±2.5 $ng \cdot L^{-1}$ immediately after exercise and returning to baseline within 1 h. [182] The impact of exercise on BNP and NT-proBNP has not been assessed in patients with PAH, where it could conceivably impact on how patients are risk stratified.

1.11.5.2 Laboratory assays variables to consider

Clinical laboratory assays have several limiting factors that can potentially impact the efficacy of patient monitoring. [183] Processing BNP and NT-proBNP samples requires analysis protocols which involve multiple stages, which when combined with resource or capacity restrictions in a hospital means that time to results can take up to several days. This limits the potential use of these biomarkers in the emergency setting, as well as the possibility of “BNP-guided therapy”, where serial measurements could be used to monitor patients and potentially guide therapy. The need for specialist laboratories prevents monitoring of patients in primary care, an approach which has been demonstrated to be potentially useful in early detection of cardiac decompensation in high-risk patients in a study of 163 patients with signs and symptoms of acute left ventricular failure (LVF). [184]

There is no standard protocol for BNP or NT-proBNP sampling and processing. Although there are suggested threshold values recommended by the different risk stratification guidelines (table 3), there will always be inconsistencies between varying tests, and there is conflicting evidence on interchangeability of results. Despite this,

studies confirm the ability of BNP and NT-proBNP to independently predict cardiovascular mortality across different thresholds, time intervals and prognostic models. [165]

1.11.6 Comparison between BNP and NT-proBNP as biomarkers in the clinical setting

BNP and NT-proBNP are both widely used in PAH risk stratification, and the ESC/ERS guidelines state that there is no clear advantage of one over the other. [11] A meta-analysis in LVF examined 48 evaluations of five different assays in 37 unique cohorts (BNP: 26 cohorts; NT-proBNP: 18 cohorts) found both molecules achieved “excellent” predictive value for excluding acute LVF at their lower cut-off thresholds. [185] However, there are clinical differences between BNP and NT-proBNP. The advantages and disadvantages of these two molecules for PAH risk stratification are summarised in table 4.

Table 4 ***Comparison of BNP versus NT-proBNP in clinical practice***

BNP	***NT- proBNP***
Active peptide, inducing compensatory mechanisms for cardiovascular injury/stress	Active function not known
Half-life ~ 22 min [169]	Half-life ~ 70 min [169]
Correlates better with pulmonary haemodynamics in PAH [186]	Correlates better with prognosis than BNP in PAH [186]
Assays use different antibodies and standard materials introducing possible inconsistencies of results between products and protocols	Assays based on the same antibodies and calibrators giving a relative consistency in results
Must not be collected in non-siliconised glass tubes [170]	Glass or plastic tubes can be used [170]
Shorter stability in storage Assay dependent Deterioration typically occurs within hours at all temperatures [170]	Longer stability in storage 7 days at room temperature 10 days at 4°C ≥ months at - 20°C [170]

Adapted from Lewis et al [164] (CC BY-NC 4.0)

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide, PAH= pulmonary arterial hypertension

BNP has been shown to correlate better with pulmonary haemodynamics in patients with renal dysfunction as it is cleared from plasma by binding to the natriuretic peptide receptor type C (NPR-C) and through proteolysis by neutral endopeptidases. Therefore only 5% is renally excreted. [186] Conversely, NT-proBNP which is mainly excreted renally, is considered more accurate for prognosis and predicting mortality because it integrates renal insufficiency and haemodynamic impairment, especially in females and younger patients. [187]

In clinical laboratory testing, the longer half-life of NT-proBNP may be beneficial if sample transportation time is high. Estimates of BNP stability recommend that it should be analysed or frozen within 4 hours, whereas NT-proBNP can reasonably be stored at room temperature for up to 2 days. [188] Therefore, it has the potential property of being used for remote monitoring of patients in the community. Another potential advantage of NT-proBNP testing over BNP testing is that all commercially available clinical laboratory immunoassays at the time of writing, including POCT assays, are based on the same antibodies and calibrators distributed by Roche Diagnostics (Rotkreuz, Switzerland), making assays relatively consistent. By contrast BNP immunoassays are more diverse, using different antibodies and standard materials. The CardioOrmoCheck study, which distributed 72 study samples to 130 Italian laboratories, found up to 50% difference in reported BNP values between assays, and these inconsistencies remained when using the same antibodies on different instruments. [167]

Cross-reactivity with proBNP-derived peptides can also affect BNP and NT-proBNP assays. The whole BNP 1–32 peptide remains intact in the C-terminal portion of proBNP, meaning that unprocessed proBNP-108 and O-glycosylated proBNP-108 can be immunoreactive in BNP assays, potentially elevating reported values. [189] Conversely, the Roche anti-NT-proBNP assay monoclonal antibodies are specific to one region of the molecule (epitope 42–46) which includes a serine at position 44 that can be glycosylated during normal post-translational processing. This can potentially reduce ligand binding and underestimate the true NT-proBNP concentration. [190] It has been suggested that extent of glycosylation at this site is influenced by pathology; increased glycosylation seen in patients with heart failure and chronic renal failure on

haemodialysis. Pre-treating samples with deglycosylation enzymes has been shown to reduce these diagnostic limitations. [191]

1.11.7 Point-of-care testing in patients with PAH

POCT for both BNP and NT-proBNP has been established for some time in the setting of left heart failure. The main advantage of POCT is it produces results in quickly with little training needed to process samples. POCT will be discussed further in the subsequent sections.

To date when searching PubMed for terms including “pulmonary arterial hypertension”, “BNP”, “NT-proBNP” and “point-of-care testing”, with no restrictions on publication date, there were a number of results for papers related to chronic heart failure and unexplained dyspnoea, but no relevant papers specifically on the subject of PAH and POCT. From other areas of medicine where POCT testing is used, one could suggest that POCT has the potential to provide a number of benefits including reduced time-to-result and time-to-diagnosis, ease of handling, along with similar analytical performance, which may lead to improved patient outcomes and clinical cost effectiveness.

1.11.7.1 Time-to-result

One of the more obvious potential benefits of BNP and NT-proBNP POCT is improved time-to-result, which usually takes 8–20 min depending on which device is used. In comparison clinical laboratory results are typically available the following day depending on the laboratory. BNP POCT assays are well established in other areas,

especially in patients with unexplained breathlessness and screening for heart failure and have been used clinically throughout the last two decades. In a study of 200 individuals evaluated for LV dysfunction, POCT could reliably predict diagnosis by echocardiogram, with an area under the curve of 0.95 and 98% specificity at a BNP level of 75 pg·mL−1. [192]

1.11.7.2 Analytical performance

The analytical performance of POCT is usually described as comparable to, or slightly lower than clinical laboratory assays, but there is general agreement that POCT is reliable enough to be used as a useful management tool. [193] In patients with LV dysfunction there are several studies that show POCT assays are comparable to clinical laboratory assays when using the same antibodies and materials. [194] There are multiple devices available for processing POCT samples for both BNP and NT-proBNP. Their comparison is detailed in the table below.

Table 5 **Comparison of 10 currently available BNP/NT-proBNP POCT devices**

Device	BNP/ NT-proBNP	Time to result	Subjects n	Correlation with clinical laboratory (unless specified)	[Refs]
Quidel Triage BNP Test (formerly Alere Heart Check)	BNP	~15 min	2260	0.95 (*versus* Siemens ADVIA Centaur) [88]	Maisel [87] (n=1586) Ro [88] (n=250) Lang [99] (n=150) Monfort [84] (n=163) De Vecchis [60] (n=111)
Quidel Triage NT-proBNP Test (formerly Alere NT-proBNP)	NT-proBNP	~20 min	100	0.94 (*versus* Roche cobas 8000)	Khezri [98]
Roche cobas h 232	NT-proBNP	⩽12 min	1887	0.97 (*versus* Roche cobas e602) [91]	Bertsch [92] (n=1591) Gils [90] (n=202) Hex [91] (n=94)
Philips Minicare BNP	BNP	⩽10 min	347	0.92 (*versus* Siemens ADVIA Centaur)	Reenen [93]
Abbott i-STAT	BNP	~9 min	400	0.98 (*versus* Siemens ADVIA Centaur) [88]	Shah [94] (n=150) Ro [88] (n=250)
Mitsubishi PATHFAST	NT-proBNP	<17 min	326	0.99 (*versus* Roche Elecsys) [96]	Peetz [96] (n=90) Zaninotto [70] (n=236)
Radiometer AQT90 FLEX	NT-proBNP	11–21 min	77	>0.99 (*versus* Roche Elecsys 2010)	Lepoutre [97]
Response Biomedical RAMP NT-proBNP	NT-proBNP	~15 min	540	0.98 (*versus* Roche Elecsys 2010)	Lee-Lewandrowski[89]
Sekisui Medical Rapidpia	BNP	<15 min	57	0.93 (*versus* SHIONOSPOT)#	Ishida [100]
Shionogi SHIONOSPOT	BNP	~16 min	57	0.93 (*versus* Rapidpia)#	Ishida [100]

Adapted from Lewis et al [164] (CC BY-NC 4.0)

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide

1.11.7.3 Ease of handling

POCT also has the potential to reduce handling errors by being simpler to use and easier to implement than clinical laboratory assays. Multiple steps in the clinical laboratory testing pathway can be reduced, including transport, storage, pre-analytical processing, result validation and return of results to treating clinician. [195]

Numerous studies have shown that POCT can be used by physicians at all grades, as well as by allied healthcare professionals and by patients or caregivers. One study found that with at least two standardised training sessions (~1.5 h total), general practitioners (GP) were successfully taught to use BNP POCT for heart failure, which

improved clinical decision making. [196] Another “untrained user study” using the Triage BNP device gave only standard user instructions to operators, who reported comparable values to clinicians experienced with the same device. [197]

1.11.7.4 Reduced costs

POCT usually has higher initial per-test costs than clinical laboratory assays, however net savings can result from reduced time-to-diagnosis/treatment and reduced hospitalisation time. [195] For example, despite slightly lower accuracy, BNP/NT-proBNP POCT was demonstrated to be reliable enough as a prognostic indicator in LVF, so could potentially lower costs by reducing the need for investigations which come at a higher cost such as echocardiography, which is often the initial investigation if deterioration is suspected. [76]

It has also been reported that NT-proBNP testing could reduce echocardiography use by up to 58%, reducing overall per patient costs by 9.4%. [198] One Spanish centre identified an optimal threshold of 280 $ng \cdot L^{-1}$ using the cobas h232 POCT device to rule out heart failure and subsequently reduced the need for echocardiography by 67%. [199] On a national scale again regarding LVF, a National Health Service (NHS) audit reported that replacing echocardiography where possible with NT-proBNP testing throughout the UK could save £1.6 million per year. [200] Therefore, it is possible that savings may be seen if POCT becomes integral to the management of patient with PH.

However, in contrast to this several studies have failed to identify a significant benefit to POCT, although they also did not conclude that POCT is disadvantageous compared with clinical laboratory testing. In a study of 711 patients seen in a dyspnoea

triage it was found that NT-proBNP POCT made no difference to time in hospital, intensive care unit admission rates or mortality. [201] A systematic review in diagnostic accuracy for acute cardiopulmonary symptoms reported limited and inconclusive evidence that GP use of POCT leads to more accurate diagnosis and improvements to clinical management. [202]

1.11.8 Establishing POCT in PAH assessment and clinical practice

The use of POCT testing in the PAH field is lacking, however in theory when examining its use in other allied areas well-established POCT devices are likely to be applicable to PAH risk stratification. In left heart failure diagnosis is typically a binary result that includes or excludes pathology depending on BNP and NT-proBNP thresholds. However, risk stratification tools used in PAH are more subtle with multiple thresholds (e.g. low, medium or high). Consequently, the use of POCT in PAH requires more research to examine the reliability of results corresponding to different thresholds.

The commonly used threshold values in heart failure due to LV dysfunction are 35 $ng{\cdot}L^{-1}$ for BNP and 125 $ng{\cdot}L^{-1}$ for NT-proBNP, or 100 $ng{\cdot}L^{-1}$ and 300 $ng{\cdot}L^{-1}$ respectively, in the acute setting. [180] High sensitivity of both clinical and POCT BNP assays at ~100 $ng{\cdot}L^{-1}$ has been reported. [203] In PAH risk stratification, the ESC/ERS guideline threshold values are higher: BNP: low <50 $ng{\cdot}L^{-1}$, intermediate 50–300 $ng{\cdot}L^{-1}$, high >300 $ng{\cdot}L^{-1}$; NT-proBNP: low <300 $ng{\cdot}L^{-1}$, intermediate 300–1400 $ng{\cdot}L^{-1}$, high >1400 $ng{\cdot}L^{-1}$ [11]. REVEAL 2.0 has similar thresholds (BNP: low <50 $ng{\cdot}L^{-1}$, intermediate 200–800 $ng{\cdot}L^{-1}$, high $\geqslant$800 $ng{\cdot}L^{-1}$; NT-proBNP: low <300 $ng{\cdot}L^{-1}$, high $\geqslant$1100 $ng{\cdot}L^{-1}$). [159]

Capillary samples have been shown to offer good correlation with venous samples in several studies, as well as acceptable reproducibility. [204] Further research is required to identify whether the specific pathophysiology of PAH affects the synthesis and secretion of biomarker molecules, and hence the reliability of BNP/NT-proBNP as a biomarker in capillary samples, especially in the context of physical exertion before sampling.

There is also the question of whether the rapid availability of results in the PAH clinic offers a similar level of added value to that seen in other pathologies. Daily, self-administered BNP POCT has been shown to be feasible and safe in outpatients. In the HABIT Trial, daily BNP tests were performed in patients with LVF and results of these tests correlated with adverse outcomes and were complementary to weight monitoring. [184] However, the value of such frequent monitoring in PAH and if it could be used to direct treatment and guide diuretic therapy is yet to be examined.

1.12 Rationale for Assessing the reliability and stability of natriuretic peptide testing in pulmonary arterial hypertension and the potential for point of care testing and remote monitoring

BNP/NT-proBNP POCT has been successfully implemented and is well established in multiple cardiovascular pathologies, including acute and chronic heart failure due to LV dysfunction. In the majority of studies, POCT has been shown to confer benefits including improved patient outcomes through reduced time-to-diagnosis and cost effectiveness. However, no studies currently exist in PAH using POCT and further

work is required to assess its potential clinical usefulness in this area. This knowledge gap is the foundation for chapter 3.

1.13 Objectives and Hypothesis of Assessing the reliability and stability of natriuretic peptide testing in pulmonary arterial hypertension and the potential for point of care testing and remote monitoring

The objectives and hypotheses of chapter 3 are detailed below.

1.13.1 Objectives

- To prospectively study the repeatability of laboratory and POCT samples for NT-proBNP and BNP in patients at rest with PAH.
- To examine the repeatability of a postal laboratory NT-proBNP and an immediately processed laboratory NT-proBNP samples.
- To assess the impact of exercise testing using the ISWT on NT-proBNP and BNP on both laboratory and POCT samples.

1.13.2 Hypotheses

- There will be no difference in value between laboratory and POCT samples for both NT-proBNP and BNP samples in patients at rest with PAH
- There will be no difference between posted laboratory NT-proBNP and immediately processed laboratory NT-proBNP samples.
- There will be no impact of exercising on NT-proBNP and BNP samples for both laboratory and POCT samples.

The results of this study form Chapter 3 of this thesis.

1.14 Chronic thromboembolic pulmonary hypertension

Having reviewed the assessment and treatment of Group 1 PAH to provide context for Chapters 3 and 4 of this thesis, the following sections provide an in-depth discussion surrounding PE and development of CTEPH (Group 4 PH), which is the subject of Chapter 4.

1.14.1 Pulmonary embolism

Venous thromboembolism (VTE), presenting as deep vein thrombosis (DVT) or PE, is globally the third most frequent acute cardiovascular syndrome behind myocardial infarction and stroke. [205] CTEPH, which is classified as group 4 PH, is a complication of pulmonary embolism. [52]

PE is a condition in which thrombus, usually embolised from the veins of the pelvis or lower limbs, obstructs the pulmonary arterial system. The incidence of PE is thought to be 60-70 per 100,000 per year [206] with a 1-year mortality of 15%. [207] PE may account for ≥300 000 deaths per year in the US, making it one of the most common causes of cardiovascular mortality. [208] In survivors, patency of the pulmonary vasculature is restored in the vast majority of patients within the first few months. [209]

1.14.1.1 Risk Factors for PE

The are several risk factors for the development of PE. PE is considered to be a consequence of the interaction between patient-related risk factors, usually permanent and setting related which are usually temporary. Table 6 lists the risk factors graded by strength of risk.

Table 6 **Risk factors for the development of PE**

Strong risk factors (OR >10)	Fracture of lower limb
	Hospitalisation for heart failure or atrial fibrillation/flutter (within previous 3 months)
	Hip or knee replacement
	Major trauma
	Myocardial infarction (within previous 3 months)
	Previous VTE
	Spinal cord injury
	Arthroscopic knee surgery
Moderate risk factors (OR 2-9)	Autoimmune diseases
	Blood transfusion
	Central venous lines
	Intravenous catheters and leads
	Chemotherapy
	Congestive heart failure or respiratory failure
	Erythropoiesis-stimulating agents
	Hormone replacement therapy (depends on formulation) In vitro fertilization
	Oral contraceptive therapy
	Post-partum period
	Infection (specifically pneumonia, urinary tract infection, and HIV)
	Inflammatory bowel disease
	Cancer (highest risk in metastatic disease)
	Paralytic stroke
	Superficial vein thrombosis
	Thrombophilia
Weak risk factors (OR <2)	Bed rest >3 days
	Diabetes mellitus
	Arterial hypertension
	Immobility due to sitting (e.g. prolonged car or air travel)
	Increasing age
	Laparoscopic surgery (e.g. cholecystectomy)
	Obesity
	Pregnancy
	Varicose veins

Adapted from the 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the ERS Konstantinides et al. [210]

Abbreviations: OR= Odds ratio, VTE= venous thromboembolism, HIV= human immunodeficiency virus

1.14.1.2 Presentation

The clinical signs and symptoms of an acute PE can be non-specific. In the majority of cases patients with a PE present with dyspnoea, chest pain, pre-syncope, syncope, or haemoptysis. In some cases, PE may be asymptomatic or discovered incidentally during diagnostic workup for another disease. [211–213]

1.14.1.3 Diagnosis

The method of choice for examining the pulmonary vasculature and thereby, investigating for PE is CTPA. It allows adequate visualisation of the pulmonary arteries down to the subsegmental level. [214–216] The Prospective Investigation On Pulmonary Embolism Diagnosis (PIOPED) II study observed a sensitivity of 83% and a specificity of 96% for CTPA in PE diagnosis. [217] Another advantage of the CTPA is that it may provide an alternative diagnosis if it is negative for PE. However, CTPA is limited by its radiation exposure in particular circumstances particularly pregnant or breast-feeding mothers, the iodine based contrast which can be problematic if allergic to iodine and those with renal failure. [210] Therefore, an alternative imaging technique can be used - ventilation/perfusion scans, which examine for ventilation-perfusion mismatching. It uses lower level of radiation and no contrast. [218] However, it has a higher rate of scans being inconclusive particularly in patient with a lower clinical suspicion of PE. [219, 220]

1.14.1.4 Treatment

Initial treatment of PE depends on the risk of immediate complications. Those with haemodynamic instability are deemed high or intermediate-high risk and as such

proceed to reperfusion treatment in the form of thrombolysis if appropriate. Patients with intermediate-low or low risk can be considered for parenteral or oral anticoagulation as initial treatment. [210] After the initial acute phase, the aim of continuing anticoagulation is to complete the treatment of the acute episode and prevent recurrence of VTE over the long-term. The length of anticoagulation has to be balanced with the risk of haemorrhage. Oral anticoagulants are highly effective in preventing recurrent VTE during treatment, but they do not eliminate the risk of subsequent recurrence after discontinuation. [221, 222] Several clinical trials have tried to address the question of length of anticoagulation following a patients first PE, it is largely agreed that all patients should receive ≥ 3 months of anticoagulation. Studies also concluded that after withdrawal of an anticoagulant, the risk of recurrence is expected to be similar if anticoagulants are stopped after 3-6 months compared with longer treatment periods (e.g 12-24 months). However, extended oral anticoagulant treatment reduces the risk of recurrent VTE by ≥ 90%, however this benefit is partially offset by the risk of bleeding. [221, 223–225]

1.14.2 Chronic thromboembolic pulmonary hypertension

1.14.2.1 Pathophysiology

The patency of the pulmonary arterial bed is restored in the majority of PE survivors within the first few months following the acute episode, therefore, no routine follow-up CTPA imaging is normally needed. [226] However, rarely, PE do not resolve and become persistent and organised, therefore developing a chronic obstructing vasculopathy. This can lead to elevation of pulmonary artery pressure and if left

untreated, the development of progressive right heart failure. This phenomenon is increasingly recognised as a long-term complication of PE and is termed CTEPH. The hallmark of CTEPH is fibrotic transformation of the pulmonary artery thrombus, causing a fixed mechanical obstruction of the pulmonary arteries. [210, 227]

1.14.2.2 Diagnostic rate of CTEPH

A recent large epidemiological analysis [228] has suggested the two-year cumulative incidence of CTEPH following a PE is 2.3% (n= 1017), with previous smaller studies estimating the incidence to be between 0.1-9.1%. [229] The wide variety of reported cumulative incidence of CTEPH is thought to be due to referral bias, the paucity of early symptoms, and the difficulty of differentiating acute PE from symptoms of pre-existing CTEPH. [230, 231] However, observed rates of CTEPH diagnosis from data reported in literature from the UK and other European countries show observed rates of CTEPH diagnosis to be 4-7 cases per million per year. [232] However, it is thought that the true diagnostic rates could be much higher than this.

1.14.2.3 Evidence for formal screening for CTEPH

Consequently, there has been interest in developing strategies to increase diagnostic rates for CTEPH and to consider early detection approaches in at risk populations. ESC/ERS guidelines on PE [233] recommend that patients should be systematically evaluated following acute PE to aid decisions with respect to long term anticoagulation strategies, modify risk-factors and co-morbidities, but also to assess for CTEPH. However, there is no consensus on how patients should be followed after an episode of acute PE. Algorithms for predicting CTEPH [234] or ruling out CTEPH [235] have been proposed, but due to lack of specificity they have not been widely incorporated into clinical practice.

1.14.2.4 Risk factors for the development of CTEPH

In an international registry, a history of acute PE was reported by 75% of patients. [236] Associated conditions and comorbidities included thrombophilic disorders, particularly antiphospholipid antibody syndrome and high coagulation factor VIII levels, cancer, a history of splenectomy, inflammatory bowel disease, ventriculo-atrial shunts, and infection of chronic intravenous lines and devices such as implantable pacemakers. [237, 238]

1.14.2.5 Clinical presentation

Diagnosing CTEPH is difficult. This is due to the clinical symptoms and signs being non-specific or absent in early CTEPH, with more significant signs of right heart failure only becoming apparent in very advanced CTEPH. Particularly early diagnosis of CTEPH remains difficult with the median time been symptoms onset and diagnosis in expert centres being 14 months. [239] The diagnosis of CTEPH can only be made after at least 3 months of adequate anticoagulation, to distinguish the condition from the acute PE. Patients have to fulfil the criteria of a diagnosis of PH based on their pulmonary haemodynamics at RHC (table 2) and have radiological evidence of (ventilation/perfusion) V/Q mismatch and signs of CTEPH on CTPA including signs such as ring-like stenoses, webs, slits and chronic total occlusions. [11]

Of note, the definition of precapillary PH has recently been revised, with a reduced PVR threshold of >2 WU from ≥3 WU. This was based on large group analysis, demonstrating the upper limit of normal is ≈2 WU, elevation above this threshold was associates with a significant and continuous increase in mortality. [240]

1.14.2.6 Treatment of CTEPH

Management of CTEPH includes a multimodal treatment approach including pulmonary vasodilator therapy, PEA and BPA. [113] Importantly, anticoagulation should continue lifelong to stop further clot formation. There are no randomised controlled trials in CTEPH with any of the approved anticoagulants, however vitamin K antagonists (VKA) and non-vitamin K oral anticoagulants (NOAC) are widely used in CTEPH. A retrospective case series from the UK and a multicentre prospective registry (EXPERT) showed comparable bleeding rates from VKAs and NOACs in CTEPH, but recurrent venous thrombo-embolism rates were higher in those receiving NOACS. [241, 242] In patients with triple positive antiphospholipid syndrome (elevated lupus anticoagulant, anti-beta-2-glycoprotein I and anticardiolipin antibodies), VKAs are recommended. [243, 244]

PEA surgery is the treatment of choice for patients with accessible pulmonary arterial lesions, [52] as surgery may normalise pulmonary haemodynamics (65% decrease in PVR) and improve functional capacity. [245] (PEA surgery is further discussed in section 1.8.1.)

BPA is an established interventional treatment in selected patients with inoperable CTEPH or persistent/recurrent PH after PEA. Data suggests improved haemodynamics (PVR decrease 49-66%), right heart function, and exercise capacity. [246–249] (BPA is discussed further in section 1.8.2.)

Medical therapy for patient with CTEPH who are awaiting surgery or have inoperable disease either due to disease distribution or co-morbidities or resistant/recurrent CTEPH are often treated with targeted medical therapies as discussed in section

1.7.1. These are largely used off-label; as the efficacy has not been proven by randomised controlled trials or registry data, [250–252] apart from riociguat which after 16 weeks, improved 6MWD and reduced PVR by 31% compared to placebo [253], subcutaneous treprostinil which showed improved 6MWD at week 24 [254] and macitentan which showed improved PVR and 6MWD versus placebo at 16 weeks and 24 weeks, respectively. [255]

1.14.3 Post PE syndrome

Post-PE syndrome due to impaired thrombus resolution and right ventricular damage encompasses a broad spectrum of disease severity, with CTEPH being the most severe manifestation. [256] Post-PE syndrome can be divided into those patients with post-PE functional limitation, post-PE cardiac impairment, chronic thromboembolic pulmonary disease (CTEPD) without PH and CTEPH. [257]

Post-PE functional limitation includes new or progressive dyspnoea and/or exercise limitations in the absence of other explanations on diagnostic investigations. The exact cause is unclear, but likely due to an element of deconditioning. A study examining 100 patients post-PE who had serial CPET and imaging, demonstrated that nearly half had reduced VO_2 peak at 1 year. With the large majority of these patients having exercise limitations in keeping with deconditioning with no patients having circulatory limitation to exercise. 60% had normal perfusion scans. [258]

Post-PE cardiac impairment is defined as incomplete restoration of RV function and is determined by an intermediate or high echo probability of PH according to the ESC criteria, with no or little chronic thromboembolic disease demonstrated on imaging. The FOCUS multicentred cohort study based diagnosis of post-PE impairment on a combination of persistent or worsening clinical symptoms, functional, biochemical, and

imaging parameters, found the cumulative incidence of post-PE impairment to be 16%. The study also found that patients with PPEI had compared to those without PPEI, higher all-cause mortality and incidence of re-hospitalisations. [228]

A group of patient post-acute PE will have chronic thromboembolic pulmonary disease (CTEPD) on imaging, but without raised pulmonary haemodynamics at RHC that meet the diagnostic criteria for PH. These patients have image findings similar to those of CTEPH and can have exercise limitation despite resting haemodynamics being in the normal range. [52] CPET performed on patients with CTEPD demonstrates reduced exercise capacity, increased dead space ventilation, inefficient ventilation and reduced O_2 pulse, in keeping with pulmonary vascular disease. [259] However, patients with CTEPD tend to have more preserved exercise capacity than patients with CTEPH. [260] Despite this, there is no defined guidance as to how to manage and treat patients with CTEPD. Although it is generally thought transition to CTEPH is uncommon, there is emerging evidence that PEA and BPA may be of benefit for these patients, in terms of improved haemodynamics, functional class and exercise capacity. [259–261]

1.15 Rationale for – Systematic pulmonary embolism follow-up increases diagnostic rates of chronic thromboembolic pulmonary hypertension: results from the ASPIRE Registry

There is no general consensus of how patients should be followed-up after having a PE, although it has been suggested by the ESC/ERS PE guidelines [210] that patients should be followed up systematically after a PE, but it is unclear the best way to do this. There is inconsistent data surrounding the true diagnostic rate of CTEPH, but it is though that conclusions made by small datasets are likely an underestimate.

Therefore, these gaps in the current literature of the impetus for examining the impact of an integrated acute pulmonary embolism pathway.

The aims and hypotheses of chapter 4 study are detailed below.

1.15.1 Objectives

- To describe the cumulative incidence of CTEPH from patients attending a dedicated PE follow-up clinic
- To describe the diagnostic rates of CTEPH in patients diagnosed in a pulmonary vascular disease unit (PVDU)
- To examine disease severity between patients from the immediate vicinity of a dedicated PVDU compared to those patients who were referred to the PVDU from the wider referral population

1.15.2 Hypotheses

- The cumulative incidence of CTEPH will be higher than previous reports
- The diagnostic rates will be higher than current literature
- Patients diagnosed with CTEPH from the PE clinic will have less severe disease compared to those who are directly referred with undefined PH

The results of this study form Chapter 4 of this thesis.

Chapter 2: Methods

Due to the format of this thesis, there is some repetition between this chapter and subsequent results chapters. However, this chapter provides a more detailed description of the methods involved in each project.

2.1 Methods chapter 3: Assessing the role of natriuretic peptides in pulmonary arterial hypertension: point of care testing and remote monitoring

2.1.1 Ethical approval

This was a sub-study of The Sheffield Teaching Hospitals Observational Study of Patients with Pulmonary Hypertension, Cardiovascular and other Respiratory Diseases (STH-ObS) research tissue bank (NHS Health Research Authority, Yorkshire & The Humber – Sheffield Research Ethics Committee, reference 18/YH/0441, IRAS 248890, HTA Licence No. 12182).

STH-ObS is a research tissue bank that collects detailed longitudinal clinical phenotypic information linked to bio-samples from patients with PH, cardiovascular disease, and other lung/respiratory diseases.

2.1.2 Inclusion and Exclusion

All Group 1 PAH patients as defined by the 6th World Symposium were included, apart from patients with a diagnosis of PAH-CHD, who were excluded along with any patients with a non-group 1 form of PH. Patients with a creatine clearance of less that <15 ml/min/m^2 were also excluded. Patients provided informed written consent prior to participation.

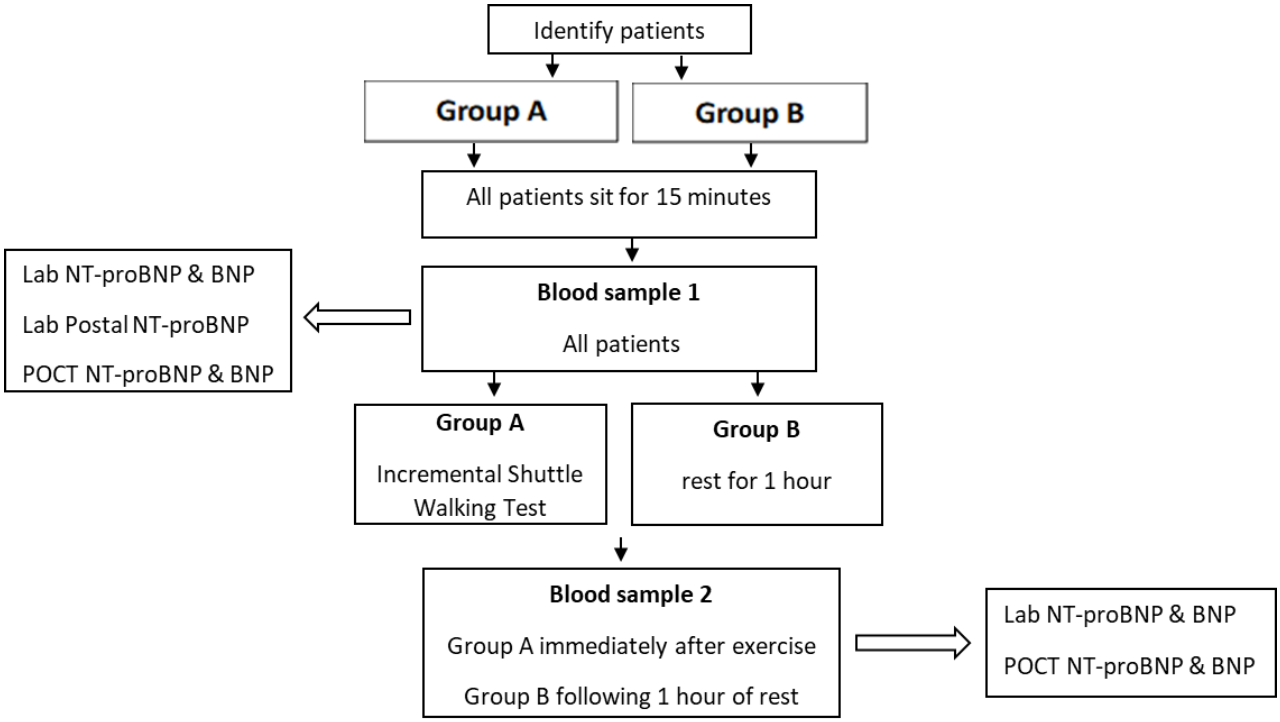
2.1.3 Diagnosis of PAH

A diagnosis of PAH was defined according to the recommendation of the 6th World Symposium on PH as mPAP >20mmHg, PAWP ≤15mmHg and PVR >240 dynes (3 Wood units) at RHC. [10]

2.1.4 The Patient pathway

The patient pathway is summarised in figure 3. Patients who were attending for a routine outpatient appointment were contacted on the telephone the week prior and given verbal information about this study. On arrival those patients willing to participate had written consent taken. Patients were allocated to either group A (exercise) or group B (rest) based on the logistics of other scheduled clinical tests during their outpatient appointment. All patients completed a rest period of 15 mins and following this the first venous blood sample was taken. Group A then proceeded to an ISWT and Group B were asked to sit and rest for 60 minutes. Following completion of the ISWT or the rest period, the second blood sample was taken. A proportion of patients reattended for a second visit as they were scheduled to return for routine follow-up during the period of the study. They were assigned to the alternative group.

Figure 3 ***The Patient Pathway***



Abbreviation: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide, Lab= laboratory, POCT= point of care test

2.1.5 Blood Sampling

Blood samples were taken at two different time points, sample 1 after the initial 15 mins of rest and the sample 2 after either exercise or a rest period of 60 minutes. Blood sampling was performed by a peripheral venous blood draw using standard venepuncture equipment. Table 7 below details the samples taken each time point.

Table 7 ***Blood sampling summary***

Blood Sample 1	Laboratory NT-proBNP
	Laboratory BNP
	POCT NT-proBNP and BNP
Blood Sample 2	POCT NT-proBNP and BNP
	Laboratory NT-proBNP and BNP

Abbreviation: BNP= b-type natriuretic peptide, NT-proBNP= N-terminal pro hormone of brain natriuretic peptide, POCT= point of care test

2.1.5.1 POC testing using the Quidel Triage MeterPro

A two-level (high/low) sample quality control (QC) was performed for every new batch of kits (a batch contains 25 test kits) and every 30 days. An internal device quality assurance was performed before use of each test kit using the Triage® Meter Pro test device. Test kits were stored between 4-8 °c as per manufacturers recommendations. Test kits were removed from the fridge and allowed to reach room temperature and remained in sealed pouches until immediately prior to use. The POCT sample blood for BNP and NT-proBNP was performed on whole blood, collected by venepuncture into an ethylenediaminetetraacetic acid (ETDA) (BD Biosciences) blood bottle. EDTA whole blood was aspirated into the pre-measured transfer pipette and the sample was dispensed into the sample port of the NT-proBNP or BNP test device. The devices for NT-proBNP and BNP were in turn inserted into the meter for analysis. See figure 4. Patient identifiers (ID) were entered, and results were printed and recorded in a custom SQL database.

Figure 4 ***The process of POCT for NT-proBNP and BNP using the Triage MeterPro point-of-care device***

Test kit in foil packet | Pipetting blood in the port | Inserting sample | Results printed

2.1.5.2 Laboratory testing of NT-proBNP and BNP

NT-proBNP samples were sent to Sheffield Teaching Hospital (STH) clinical chemistry laboratory for NT-proBNP analysis. These samples were processed using a Roche COBAS 8000 (c702) modular analyser. Processing for BNP is not routine at STH, therefore samples were frozen immediately after being taken, stored at -80 C and transported on dry ice to Barnsley District General Hospital clinical chemistry laboratory for analysis. These samples were again processed using a Roche COBAS 8000 modular analyser.

2.1.6 Postal NT-proBNP

Postal venous blood samples were taken at the same time as the immediately processed NT-proBNP into a standard EDTA blood bottle, however instead of being sent straight to the laboratory, the blood bottle was placed securely in UN3373 compliant packages, which were sealed and addressed to the hospital biochemistry laboratory. The samples were taken to the hospital post room and were sent the same day via external mail. Once receiving these boxes in the clinical chemistry laboratory, the samples were then processed as per the normal protocol. Time of postal samples

received and processed was recorded using the standard investigation reporting system (ICE reporting) used throughout the hospital.

2.1.7 Incremental Shuttle Walking Test

ISWT were undertaken as described by Singh et al. [262] Patients were asked to complete a 10 m length keeping in time with an audible bleep. Level one consists of three lengths (30 m), and each subsequent level adds one extra length to the preceding level. The initial speed is a slow walk (0.50 m/s), increasing incrementally every level to a maximum speed of 2.37 m/s at level 12. Each level takes 1 minute to complete, and the test finishes at the end of level 12, a distance of 1020 m. The patient continues until they were too breathless or unable to keep up with the required pace. Patients who were unable to perform an ISWT because of breathlessness were ascribed an ISWD of 0 m.

2.1.8 Risk Stratification

Thresholds from the COMPERA 2.0, 4-strata risk assessment tool [160] were used to define low (BNP <50ng/L, NT-proBNP <300ng/L), intermediate-low (BNP <50-199ng/L, NT-proBNP 300-649ng/L), intermediate-high (BNP 200-800ng/L, NT-proBNP 650-1100ng/L) and high-risk groups (BNP >800ng/L, NT-proBNP >1100ng/L).

2.1.9 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v26 (SPSS, Chicago, IL, USA) and MedCalc, version 19.4 (MedCalc Software, Ostend, Belgium). Continuous variables were described by mean ± standard deviation (SD).

A number of statistical tests were applied to assess reliability between the laboratory and POCT assays. Passing-Bablok regression was used to examine agreement between laboratory and POCT, this method allowed for the estimation of agreement and possible systematic bias between the two variables. It provided a useful binary measure (equivalent or not equivalent) on the basis of the 95% CI of the intercept including zero and the 95% CI of the slope and spanning 1. [263] Bland-Altman plots were also utilised to provide another measure of agreement with a visual representation evaluating bias between the mean differences in results between the two assays and estimated an agreement interval, within which 95% of the differences lie. However, Bland-Altman plots only define the intervals of agreement and do not indicate if those limits are acceptable or not. Therefore, clinical interpretation is needed to decide what level of discrepancy between the two variables in acceptable. [264] The intraclass correlation (ICC) was used to examine the correlation between laboratory and POCT samples. [265] Guideline thresholds have been published to aid interpretation of the results which lie between 0-1, with ICC values over 0.90 deemed as excellent agreement. [266] Also used was Lin's concordance coefficient (CCC) which quantified agreement by measuring how well the observation conforms to the gold standard observation (laboratory sample in this study). The CCC ranges from -1 to 1 with perfect agreement being 1, interpretation thresholds set as >0.99 =almost perfect, 0.95 to 0.99 = substantial, 0.90 to 0.95 = moderate and <0.90 = poor. Paired t-tests or Wilcoxon tests were used when examining the difference between 2 variables. A p value <0.05 was deemed statistically significant.

2.2 Methods for Chapter 4 – Systematic pulmonary embolism follow-up increases diagnostic rates of chronic thromboembolic pulmonary hypertension: results from the ASPIRE Registry

2.2.1 Ethical approval

Ethical approval for this study was obtained through the ASPIRE (Assessing the Severity of Pulmonary Hypertension In a Pulmonary Hypertension Referral Centre) registry (REC 22/EE/0011).

The ASPIRE Registry is an extensive database, where confidential data is stored from all patients evaluated for suspected pulmonary vascular disease attending Sheffield Teaching Hospitals NHS Foundation Trust. Data stored includes demographic, standard clinical physiological measurements including spirometry results and exercise test data, laboratory blood tests, imaging, RHC metrics and medications. Data are only collected during the course of normal clinical care.

2.2.2 Inclusion and exclusion

Consecutive patients who were admitted to Sheffield Teaching Hospitals NHS foundation Trust with an acute episode of PE, upon discharge were referred to a dedicated PE follow-up clinic. All patients who attended this acute PE follow-up clinic between March 2010 and March 2020 were included. Consecutive patients diagnosed with CTEPH, as defined below, from the ASPIRE Registry (Assessing the Spectrum of Pulmonary Hypertension Identified at a Referral Centre) at the SPVDU over the same period were also included.

2.2.3 The PE Service

All Sheffield residents discharged from STH with a new diagnosis of acute PE are referred to Sheffield PE clinic. This PE clinic was established in 2010. Patients who are referred were assessed within 1 week of discharge by an anticoagulation specialist nurse led telephone clinic which included a clinical assessment and malignancy screening. If concerns were identified at this point, patients were discussed at a thrombosis multidiscipline team (MDT). Otherwise, patients were reviewed 3-4 months after their acute PE in a joint PE follow-up clinic, with a respiratory physician specialising in PE and a haematologist specialising in thrombosis. Patients were individually evaluated with respect to risk of VTE recurrence, malignancy screen, appropriate duration of anticoagulation and assessment for possible CTEPH (Figure 13). CTEPH screening included a review of the clinical presentation and diagnostic imaging, assessment of symptoms and review of risk factors for CTEPH. In selected cases, where CTEPH was felt to be a possibility, further investigation for CTEPH including an assessment of further lung imaging and perfusion and assessment of pulmonary artery pressure by an echocardiogram is normally performed in the first instance. Patients with suspected PH were referred to the Sheffield PH referral centre where they underwent systematic evaluation with multimodality imaging (lung scintigraphy, CT pulmonary angiography, CMR) with MR angiography and where there remained diagnostic doubt digital subtraction angiography) and RHC. Patients who were recovered from their acute PE or investigated and found not to have CTEPH were discharged from the PE services with advice to represent for assessment if they developed symptoms of CTEPH. Unless the patient declined, all cases of CTEPH

were referred to the UK national referral centre for PEA and BPA (Royal Papworth Hospital). [146]

2.2.4 CTEPH definition

CTEPH was defined according to the recommendations of 2022 ESC/ERS PH guidelines and required supportive imaging, a mPAP >20 mmHg at RHC, with other causes of PH excluded. In occasional cases RHC data was not available, for example in patients who declined or where co-morbidities made invasive investigations inappropriate. In these cases, a diagnosis of CTEPH was made by multimodality imaging and expert opinion.

2.2.5 Population data

Rates for annual incidence were based on a mean population of the city of Sheffield, derived from the mean of consecutive census years between 2011 and 2021 from the department of national statistics census data, giving a figure of 554,600. The Sheffield PH referral centre is part of a UK national network, adhering to annually audited and published standards of care, and covers a referral population of 15–20 million.

2.2.6 Patient Groupings

All patients diagnosed with CTEPH during the study period were divided into 3 groups according to the origin of referral: Sheffield PE clinic, Sheffield residents referred with suspected PH who had not attended the PE clinic, and non- Sheffield residents referred with suspected PH from the wider referral area. Sheffield residents were defined as those who lived in a postcode area of the City of Sheffield as defined by the Sheffield City council. For the 3 patient groups, demographic, investigations, and survival data were retrieved from the ASPIRE registry.

2.2.7 Radiological Analysis

For patients diagnosed with CTEPH attending the Sheffield PE clinic, CTPAs were retrieved and reviewed by a consultant radiologist experienced in pulmonary vascular disease. The presence or absence of CT features predictive of the presence of PH and CTEPD were recorded. Where all 3 features of PH were present; PA size≥30mm, right ventricular outflow hypertrophy ≥6mm and right ventricular:left ventricular ratio ≥1 and 2 of 4 features of CTEPD (dilated bronchial arteries, arterial webs or bands, attenuated or occluded vessels and mosaic parenchymal perfusion pattern) were present, the patient was diagnosed as having CTEPH at the time of the initial presentation. A control group of randomly selected patients who were not diagnosed during follow-up with CTEPH were also examined for features of PH and CTEPD at the time of the index PE.

2.2.8 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics v26 (SPSS, Chicago, IL, USA). Continuous variables were described by mean ± SD and data that was not normally distributed are shown as median ± interquartile range (IQR). Kruskal-Wallis statistical test with Bonferroni correction was used to compare the 3 groups. Event (death)- free survival from the date of diagnosis was estimated using the Kaplan-Meier method with comparison between groups performed by the log-rank test. A p-value <0.05 was deemed statistically significant.

Chapter 3: Assessing the reliability and stability of NT-proBNP and BNP testing in pulmonary arterial hypertension and the potential for point-of-care testing and remote monitoring

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Abstract

Introduction BNP and NT-proBNP are important biomarkers and key to risk stratification in PAH. However, prolonged processing times mean results are rarely available at the time of clinical assessment and their utility in remote monitoring is unknown. We tested the reliability of NT-proBNP/BNP POCT in PAH patients and investigated the stability of NT-proBNP in posted blood samples with delayed analysis, to simulate remotely obtained blood samples.

Method Patients with Group-1 PAH were recruited prospectively. Blood samples were taken at two time-points, pre- and post-exercise, for laboratory and POCT NT-proBNP/BNP. A separate sample was returned to the laboratory by post.

Results 41 patients were enrolled with a total of 56 study visits. The coefficient of variation (CV) for NT-proBNP laboratory samples was 4.7% (n=18) and 8.7% (n=19) for laboratory BNP. POCT NT-proBNP CV was 9.7% (n=19) and 9.2% (n=19) for POCT BNP. Comparing NT-proBNP laboratory vs. POCT testing (n=50) provided equivalent test results according to Passing-Bablok (slope=1.08, CI0.97-1.19, intercept=-18.22, CI-41.6-4.5) and ICC=0.97. However, for laboratory vs. POCT BNP (n=49), Passing-Bablok showed non-equivalence (slope=1.24, CI=1.11-1.31, intercept=-5.11, CI-9.4- -0.46), ICC=0.96. POCT NT-proBNP/BNP correctly classified 94% and 88% of cases, respectively against COMPERA 2.0 4-risk-strata thresholds. NT-proBNP postal laboratory samples and immediately processed NT-proBNP laboratory samples showed good agreement.

Conclusion POCT provides a rapidly accessible and reliable alternative to laboratory NT-proBNP/BNP. Time-delayed analysis of postal laboratory NT-proBNP

demonstrated reliable results highlighting its potential in remote monitoring of patients with PAH.

Key message

NT-proBNP and BNP levels are important in the risk stratification of patients with PAH, but results are rarely available at the time of face-to-face appointments. We have demonstrated that POCT for both NT-proBNP and BNP are reliable tools in providing quick, easy, and consistent results. In addition, samples posted to a laboratory and tested for NT-proBNP produced repeatable reliable results highlighting their potential in the remote monitoring of patients with PAH.

Introduction

NT-proBNP and BNP are important prognostic biomarkers in PAH. [172, 173, 267] NT-proBNP and BNP are natriuretic proteins that are primarily released from the cardiomyocytes in response to mechanical load and ventricular wall stress. [268] In PAH, the rising pulmonary artery pressures lead to dysfunction of the RV and eventually right heart failure and death. [13] Right ventricular function is thought to be the primary determinant of survival in PAH. CMR predicts clinical worsening and prognosis [81] and MRI measures of RV function can be used to aid risk stratification [269], however CMR is costly and is challenging to perform remotely. NT-proBNP and BNP are released by the ventricles in proportion to the extent of cardiac wall stress and have been used as a surrogate marker of RV function. [169, 170] Treatment options for PAH have expanded in the last decade, leading to an improved prognosis [270] and categorising patients according to risk of deterioration informs the initial treatment strategy and subsequent timing of treatment escalation. Several risk stratification tools are commonly used in categorising patients with PAH including the 2022 ERS/ESC risk stratification and COMPERA 2.0. [11, 159, 271–275] Integral to all is the inclusion of NT-proBNP and BNP.

NT-proBNP and BNP samples are collected by venepuncture, and the results are rarely available to the clinician on the same day. Alternative methods of testing NT-proBNP and BNP have been studied in patients with left heart disease, where a threshold level of NT-proBNP or BNP is used to detect early cardiac decompensation in high-risk patients.

POCT for both BNP and NT-proBNP has been established in this setting for some time and reduces time to results. [268] However, information on use of POCT for NT-

proBNP and BNP in the setting of PAH is lacking. There are also limited available data concerning the repeatability of both laboratory and POCT measurements of NT-proBNP and BNP in patients with PAH. Furthermore, no data are available to assess the reliability of POCT across the wide range of values encompassed by PAH risk stratification tools with three or four strata. As a result, further research is needed to investigate the reliability of POCT results associated with various threshold levels in the context of PAH.

Patients with PAH frequently travel long distances to pulmonary hypertension referral centres for face-to-face assessments. During the COVID-19 pandemic remote assessment frequently replaced face-to-face appointments and identified the need for objective methods for remote monitoring of PAH in addition to a symptomatic assessment. [276, 277] As NT-proBNP can provide a reflection of RV dysfunction, [173] and is more stable than BNP, [187] it could have a role in remote monitoring of patients with PAH and would complement remote assessment of exercise capacity. However, there are limited data on factors that may influence NT-proBNP levels in a remote setting, such as pre-processing time delay and exercise. The first aim of this study was to examine the agreement between POCT and laboratory NT-proBNP and BNP across the spectrum of COMPERA 2.0 stratification values. Secondly, we aimed to examine the stability of NT-proBNP in a potential role of remote monitoring in PAH.

Methods

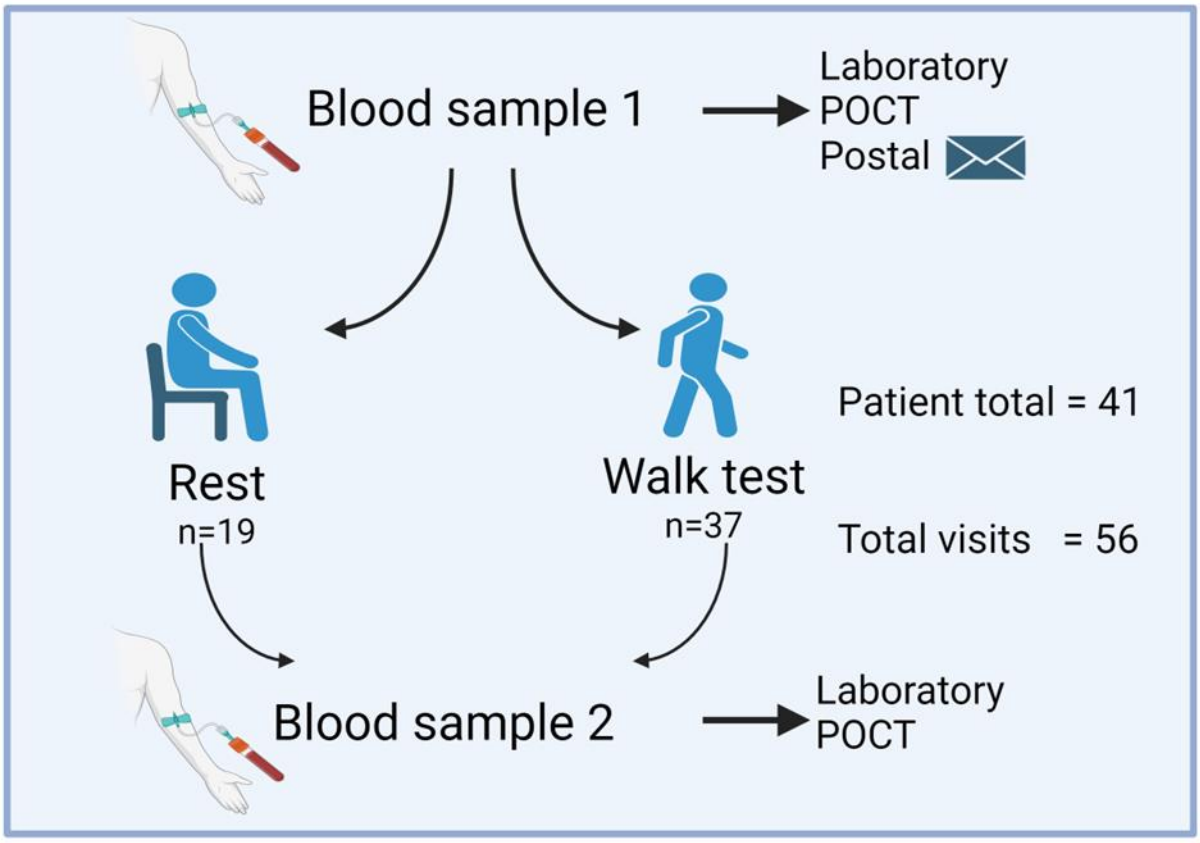
Inclusion and exclusion

Patients with Group 1 PAH were included, with the exception of PAH-CHD. PAH was defined according to the 6th World Symposium on PH: mPAP >20 mmHg, PAWP ≤15 mmHg and PVR >240 dyn.s.cm^{-5} (3 Wood units) and had undergone systematic evaluation including multimodality imaging as previously described. [278] Those with a creatinine clearance of less than <15 ml/min/m^2 were excluded. Patients gave informed consent to participate in the study, which was an approved sub-study of STH-ObS research tissue bank (NHS Health Research Authority, Yorkshire & The Humber – Sheffield Research Ethics Committee, reference 18/YH/0441, IRAS 248890, HTA Licence No. 12182).

The Patient pathway

The patient pathway is summarised in Figure 5. Patients were allocated to either rest or exercise based on other scheduled clinical tests. All patients completed an initial rest period of 15 mins before the first venous blood sample was taken. The exercise group then proceeded to an ISWT and the rest group were asked to sit and rest for 60 minutes. The second venous blood sample was taken immediately following completion of the ISWT or the rest period. A proportion of patients reattended for a second visit and were assigned to the alternative group (exercise or rest) from their first visit. Although the 6MWD is more commonly used in PH centres, the ISWT is the preferred mode of assessment of exercise capacity in Sheffield pulmonary vascular disease unit.

Figure 5 ***The Protocol***



There were 56 patient visits, with 26 participants attending for one visit and 15 attending for two visits. Blood was taken before and after a period of rest, or before and after an incremental shuttle walk test, with samples analysed in a laboratory or by POCT as indicated.

Abbreviations: NT-proBNP= N-terminal Pro Hormone of B-type Natriuretic Peptide, BNP= B-type Natriuretic Peptide, POCT= point-of-care test.

POC testing using the Quidel Triage® MeterPro®

A two-level (high/low) sample QC was performed for every new batch of kits (a batch contains 25 test kits) and every 30 days. An internal device quality assurance was performed before use of each test kit. Test kits were stored between 4-8 °C and allowed to reach room temperature prior to use. POCT was performed on whole blood drawn into blood bottles containing ETDA (BD Biosciences). Blood was aspirated into a transfer pipette and the sample was dispensed into the sample port of the POCT

test kits. The test kits for NT-proBNP and BNP were in turn inserted into the MeterPro® device for analysis.

Laboratory testing of NT-proBNP and BNP

NHS laboratory NT-proBNP analysis was performed on serum (BD Vacutainer, SST™II advance) using a Roche COBAS 8000 (c702) system. Aliquots of EDTA plasma were immediately frozen at -80 °C for BNP analysis, as a single batch, using a Roche COBAS 8000 modular analyser system.

Postal Laboratory Testing for NT-proBNP

Whole blood collected into serum tubes were either immediately delivered to the clinical chemistry laboratory or packaged in secure UN3373 compliant packaging and mailed back via external postal service. Upon receipt, NT-proBNP level was analysed using a Roche COBAS 8000 system.

Incremental Shuttle Walking Test

Incremental shuttle walking tests was undertaken as previously described. [75] Patients were asked to complete a 10-m length keeping in time with an audible bleep. Level one consists of three lengths (30 m), and each subsequent level added one extra length to the preceding level.

Risk stratification

Thresholds from the COMPERA 2.0, 4-strata risk assessment tool [160] were used to define low (BNP <50ng/L, NT-proBNP <300ng/L), intermediate-low (BNP <50-

199ng/L, NT-proBNP 300-649ng/L), intermediate-high (BNP 200-800ng/L, NT-proBNP 650-1100ng/L) and high-risk groups (BNP >800ng/L, NT-proBNP >1100ng/L).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v26 (SPSS, Chicago, IL, USA) and MedCalc, version 19.4 (MedCalc Software, Ostend, Belgium). Parametric continuous variables were described by mean ± SD, and non-parametric by median and IQR. The repeatability of measurements taken 60 minutes apart, while the patient rested, were calculated for laboratory and POCT assays. The SD of the two values was divided by the mean and multiplied by 100 to give a CV. Bland-Altman plots, Passing-Bablok regression, ICC and Lin's CCC were used to describe the relationship between POCT and laboratory test results. Paired t-tests or Wilcoxon tests were used when examining the difference between two variables. A p value <0.05 was deemed statistically significant.

Results

Between April 2021 and October 2022, 41 patients were recruited; demographic data are provided in Table 8. There were a total of 56 visits (15 patients had 2 visits within the study period). There was female predominance (71%) with a median age of 55.8 years. The majority of patients had idiopathic PAH (83% IPAH), with PAH in association with connective tissue disorders and heritable PAH making up a smaller proportion of patients (12% and 5% respectively). Ten POCT results for NT-proBNP were excluded due to one batch of kits failing the quality control process. All BNP POCT were processed successfully. One NT-proBNP and one BNP laboratory sample

result was missing. A larger proportion of patients were recruited to “exercise” (n=37) in comparison to “rest” (n=19).

Table 8 ***Patient Demographics***

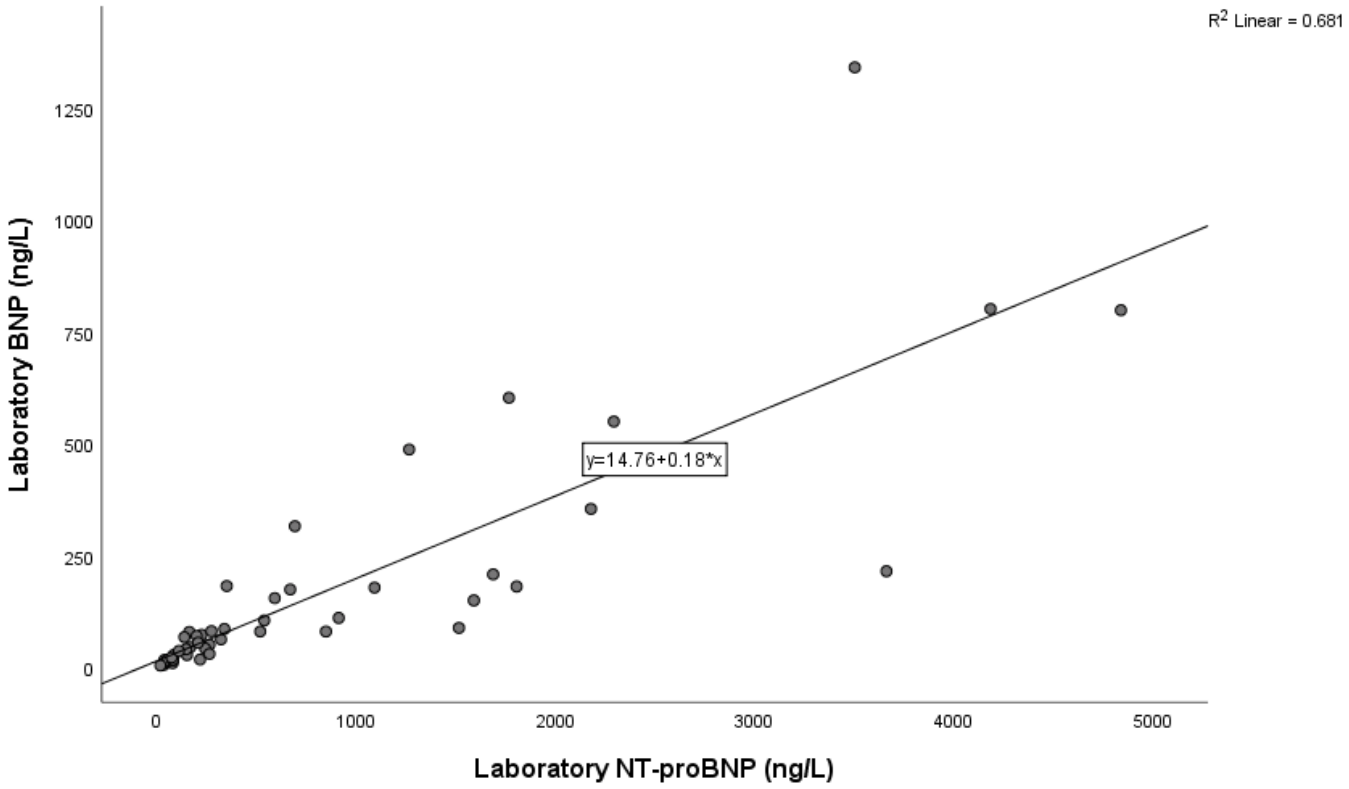
Total number of patients	41	
Total number of visits (rest/exercise)	56 (19/37)	
Female (n (%))	29 (71)	
Age (Years) at first visit	55.8	
WHO FC I/II/III/IV (%)	5/37/58/0	
Subtype of Pulmonary Arterial Hypertension (n (%))	Idiopathic	34 (83)
	Connective Tissue Disease^	5 (12)
	Heritable	2 (5)
Co-morbidities	Lung disease*	8 (20)
	Thromboembolism	5 (12)
	Obstructive Sleep Apnoea	5 (12)
	Thyroid Disease	5 (12)
	Hypertension	5 (12)
	Intracardiac shunt	5 (12)
	Obesity	4 (10)

*Lung disease = asthma 10%, COPD 7%, bronchiectasis 2%
^ Connective tissue disease = 12% of which, 10% systemic sclerosis and 2% dermatomyositis
Abbreviations: WHO FC= World Health Organisation Functional Class

Intra-assay variability

For laboratory samples the CV was 4.7% (n=18) for NT-proBNP and 8.7% (n=19) for BNP samples, while for POCT the CV was 9.7% (n=19) for NT-proBNP and 9.2% (n=19) for BNP. In addition, there was a strong correlation between NT-proBNP and BNP (r^2=0.95, p=<0.001) (figure 6).

Figure 6 ***Correlation between laboratory NT-proBNP and BNP***



Spearman's rank coefficient for laboratory NT-proBNP and BNP, r^s = 0.945, p=<0.01, (n=49)

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide

Performance of POCT versus laboratory NT-proBNP

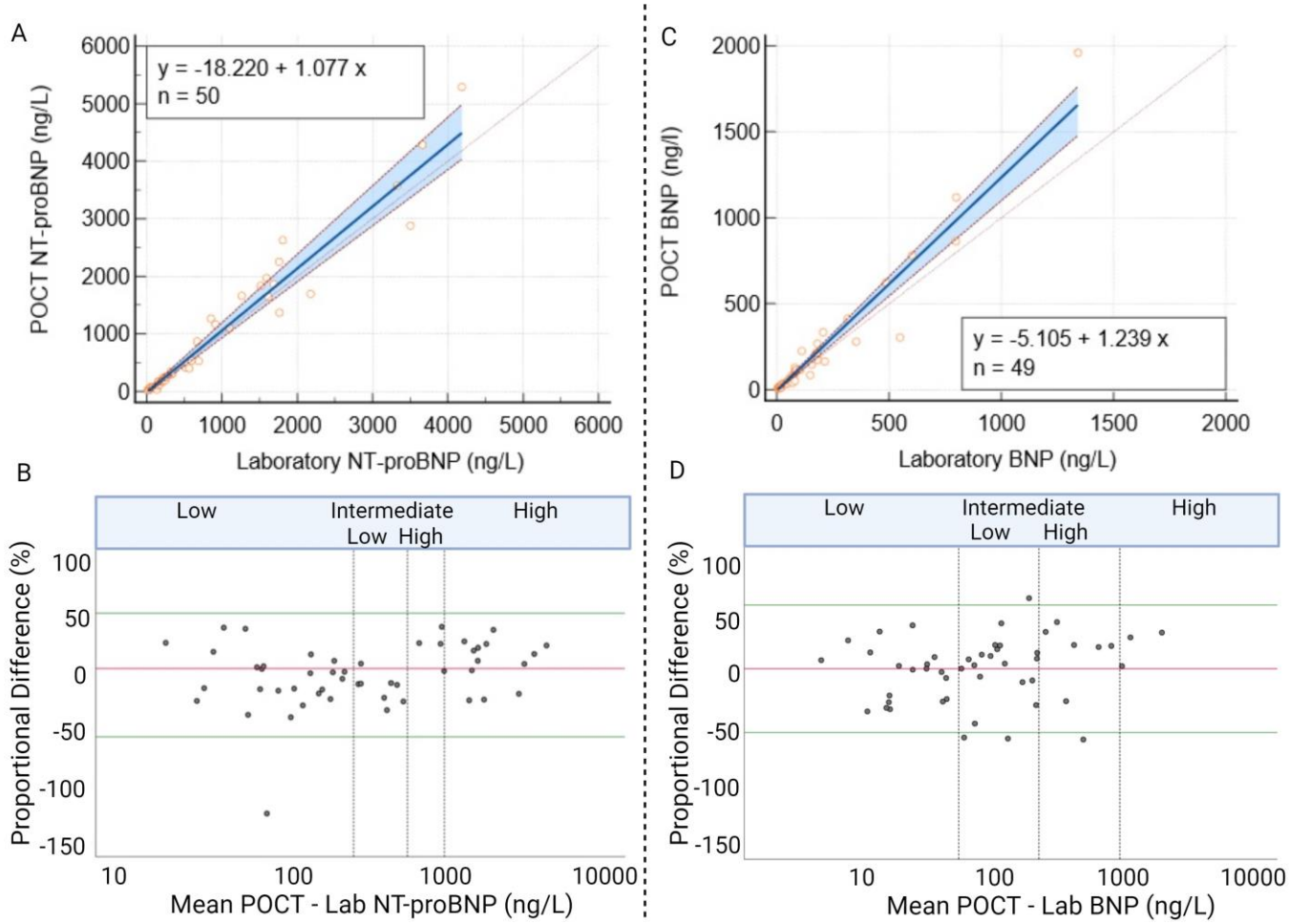
The relationship between POCT and laboratory NT-proBNP results (n=50) revealed an ICC of 0.97 (95% CI 0.95-0.98). Passing-Bablok regression demonstrated an estimated slope of 1.08 (95% CI 0.97-1.19), intercept of -18.22 (95% CI -41.6-4.5) indicating measures were equivalent (Figure 7A). When examining the agreement between absolute values of POCT and laboratory NT-proBNP, there was evidence of proportional bias (Figure 8A). Therefore Bland-Altman plots were constructed using percentage differences (Figure 7B), and this displayed a mean bias of -2.87 ± 27.98%, and limits of agreement of 57.7 to -51.95%. Lin's CCC was 0.97 (95% CI = 0.95-0.98) indicating 'substantial' concordance (Table 9). The performance of NT-proBNP POCT was examined against the COMPERA 2.0 4-strata risk scoring tool, dividing 1-year mortality risk into low/intermediate-low/intermediate-high/high. NT-proBNP POCT identified 94% patients into the correct risk category, based on the corresponding reference laboratory NT-proBNP.

Performance of POCT versus laboratory BNP

The agreement between POCT and laboratory BNP (n=49), was not equivalent as demonstrated by the Passing-Bablok estimated slope of 1.24 (95% CI 1.11-1.31), intercept of -5.11 (95% CI -9.4 to -0.46), as the 95% confidence intervals for slope did not include 1 (Figure 7C). The Bland-Altman plot of percentage difference demonstrated a mean bias of 5.4 ± 28.86%, with limits of agreement -51.24% to 62.04% (Figure 7D). Examining the agreement again between absolute values of POCT and laboratory NT-proBNP, there was evidence of proportional bias (Figure 8B). Lin's CCC was 0.93 (95%CI 0.90-0.95) demonstrating moderate concordance and the ICC was 0.96 (95% CI=0.94-0.98) (table 9). When focusing on BNP

performance in the context of the COMPERA 2.0 4-strata risk scoring tool BNP POCT identified 86% of patients into the correct risk group.

Figure 7 ***Performance of POCT versus laboratory NT-proBNP and BNP***

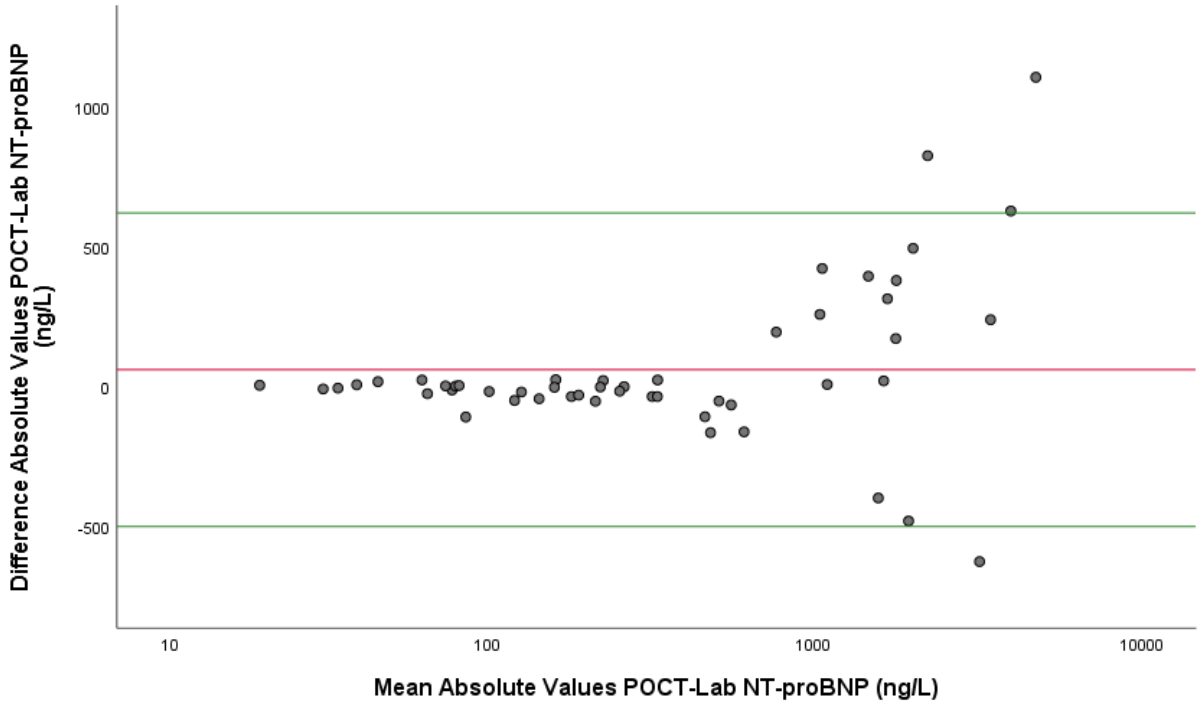


Performance of POCT versus laboratory NT-proBNP n=50. (A) Passing-Bablok regression (slope=1.08, 95% CI 0.97-1.19, intercept of -18.22, 95% CI -41.6-4.5) (B) Bland-Altman, mean bias=-2.87 ± 27.98%, limits of agreement of -57.7 to – 51.95%. Performance of POCT versus laboratory BNP. (C) Passing-Bablok regression (slope=1.24, 95% CI 1.11 – 1.31, intercept= -5.11, 95% CI -9.4 to -0.46, p=0.42). (D) Bland-Altman, mean bias 5.4% ± 28.86%, limits of agreement -51.24% – 62.04%). X axis on a logarithmic scale.

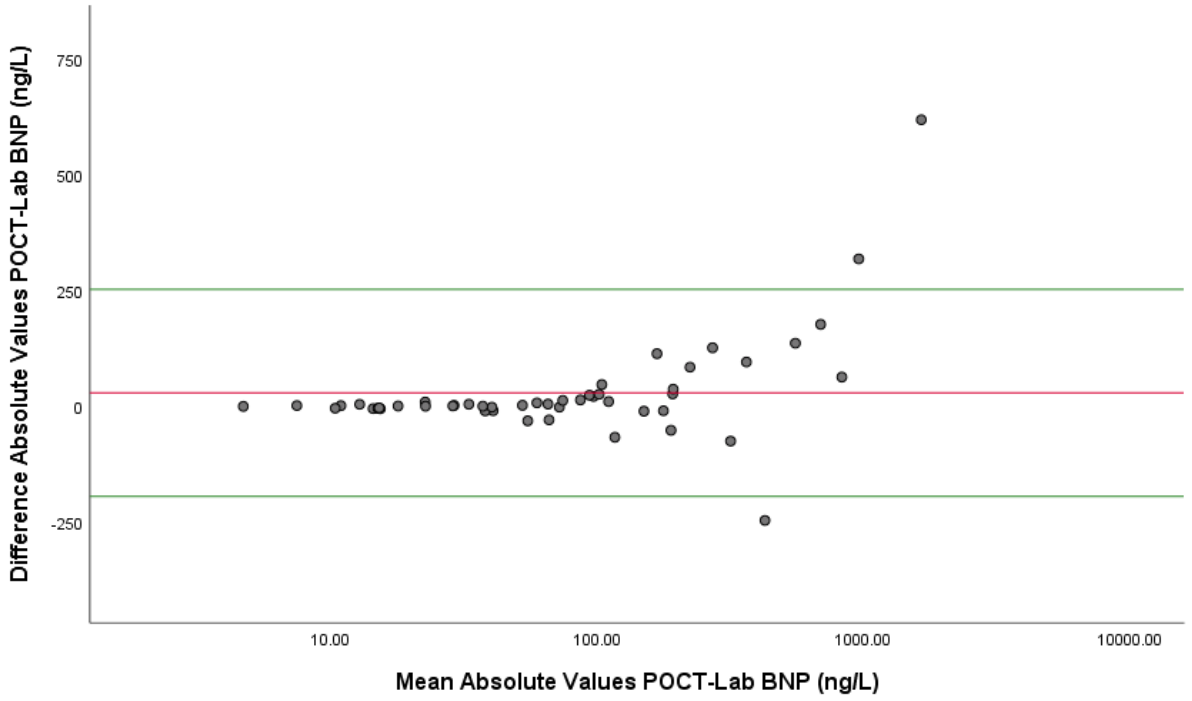
Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide, POCT= point of care test

Figure 8 ***The agreement between absolute values of POCT and laboratory for NT-proBNP and BNP***

9A.



9B



(A) Bland-Altman demonstrating the agreement between the absolute values of laboratory NT-proBNP, mean = 60.9±285.4, limits of agreement 620.3 to -498.5 to -20.64 (n=50). (B) Bland-Altman for laboratory BNP, mean= 29.8±114.01, limits of agreement 253.3 to -193.7. (n=49).
Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide, POCT= point of care test

Table 9 ***Summary table of statistics for Laboratory versus POCT for NT-proBNP and BNP***

Statistical Test		POCT vs. Laboratory NT-proBNP n=50		POCT vs. Laboratory BNP n=49	
Passing-Bablok regression	Slope	1.08	95% CI 0.97-1.19	1.24	95% CI 1.11-1.31
	Intercept	-18.22	95% CI -41.6-4.5	5.11	95% CI -9.4- -0.46
Bland-Altman	Mean bias	-2.87		5.4	
	SD of bias	27.98		28.86	
	Limits of agreement	-57.7 to 51.95		-51.24 to 62.04	
Intra-class coefficient		0.98	95% CI 0.97-0.99	0.96	95% CI 0.94-0.98
Lin's Concordance coefficient		0.98	95% CI 0.97-0.99	0.93	95% CI 0.90-0.95

Abbreviations: BNP= brain natriuretic peptide, CI= confidence interval, NT-proBNP= N-terminal prohormone of brain natriuretic peptide, POCT= point of care test, SD= standard deviation

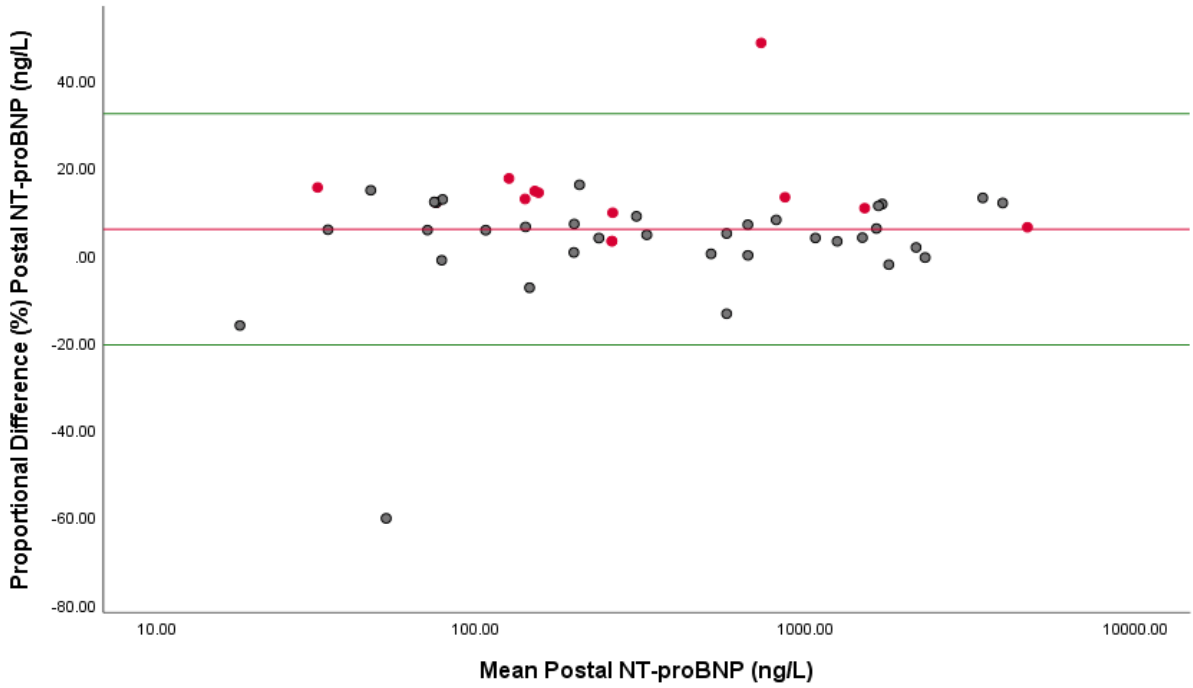
Postal Laboratory NT-proBNP

Of the 56 laboratory NT-proBNP samples packaged and sent by post, 88% of samples were processed. Five samples did not return to the laboratory and 1 sample was returned, but was discarded in error. Postal samples were received by the laboratory 2.04 (IQR 2.35) days after being sampled. Of the remaining laboratory NT-proBNP postal samples, 48 of 50 were analysed, 2 samples did not have a corresponding reference laboratory NT-proBNP sample to allow comparison.

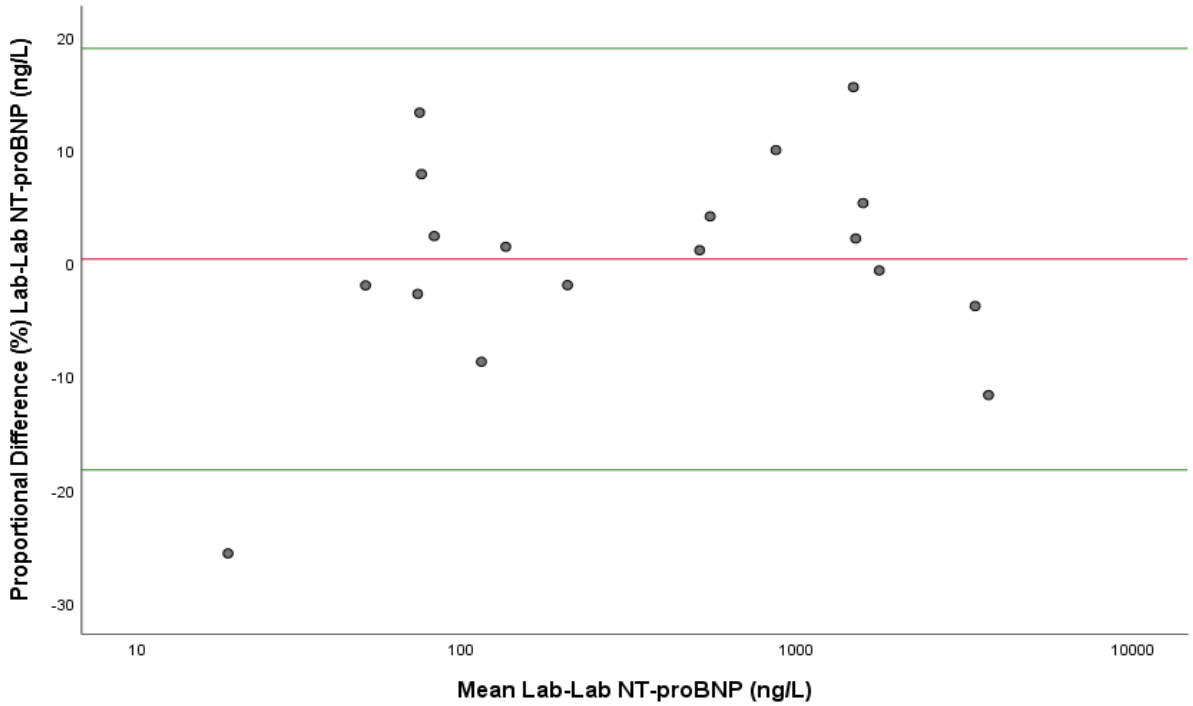
Assessment of agreement between reference NT-proBNP and postal NT-proBNP samples (n=48) demonstrated the proportional % difference demonstrated a mean bias of 5.83 ± 13.51% with limits of agreement -20.64% to 32.3% (Figure 9A). This is in comparison to agreement between initial laboratory NT-proBNP and repeated laboratory NT-proBNP (n=18) where the proportional percentage difference demonstrated a mean bias of 0.37 ± 9.51% with limits of agreement -18.27% to 19.01 (Figure 9B). There was a trend towards increasing proportional difference between results as the time delay to processing increased although this did not reach statistical significance (R^2=0.186, p = 0.09).

Figure 9 ***Performance of postal NT-proBNP versus laboratory NT-proBNP***

A.



B.

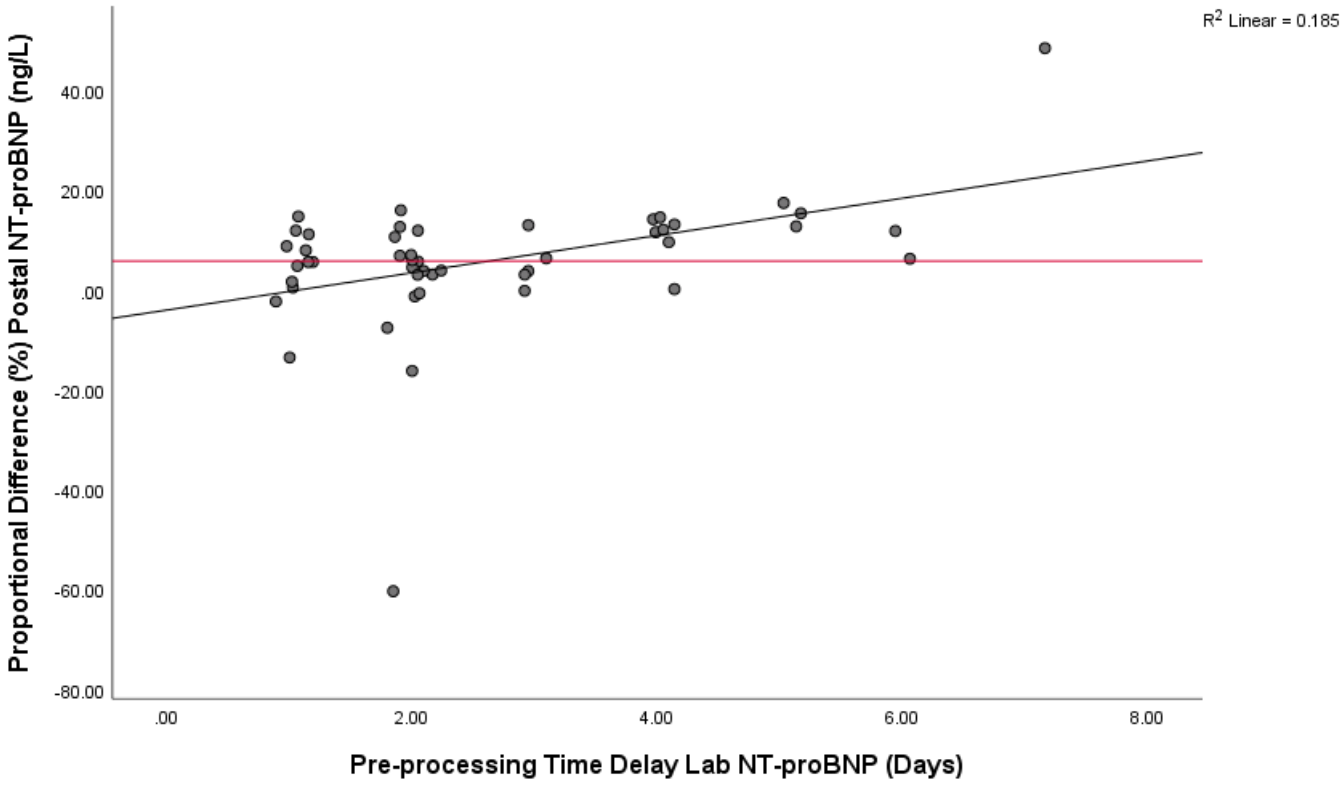


(A) Bland-Altman demonstrating the agreement between immediately processed laboratory NT-proBNP and postal laboratory NT-proBNP, mean difference = 5.83±13.51%, limits of agreement 32.3 to -20.64 (Highlighted red point are those processed >4days) (n=49). (B) Bland-Altman for laboratory NT-proBNP taken at 2 difference time points (n=18), mean= 0.37±9.51%, limits of agreement 19.01 to -18.27. X axis on a logarithmic scale.

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide

Results remained accurate with less than 20% proportional difference up to a pre-processing time delay of 6 days, although the low number of postal NT-proBNP samples returned after 5 days limits our ability to draw firm conclusions (Figure 10).

Figure 10 ***Performance of delayed processing of NT-proBNP over time***



Proportional difference between immediately processed laboratory NT-proBNP and postal laboratory NT-proBNP over time (n=48).

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide

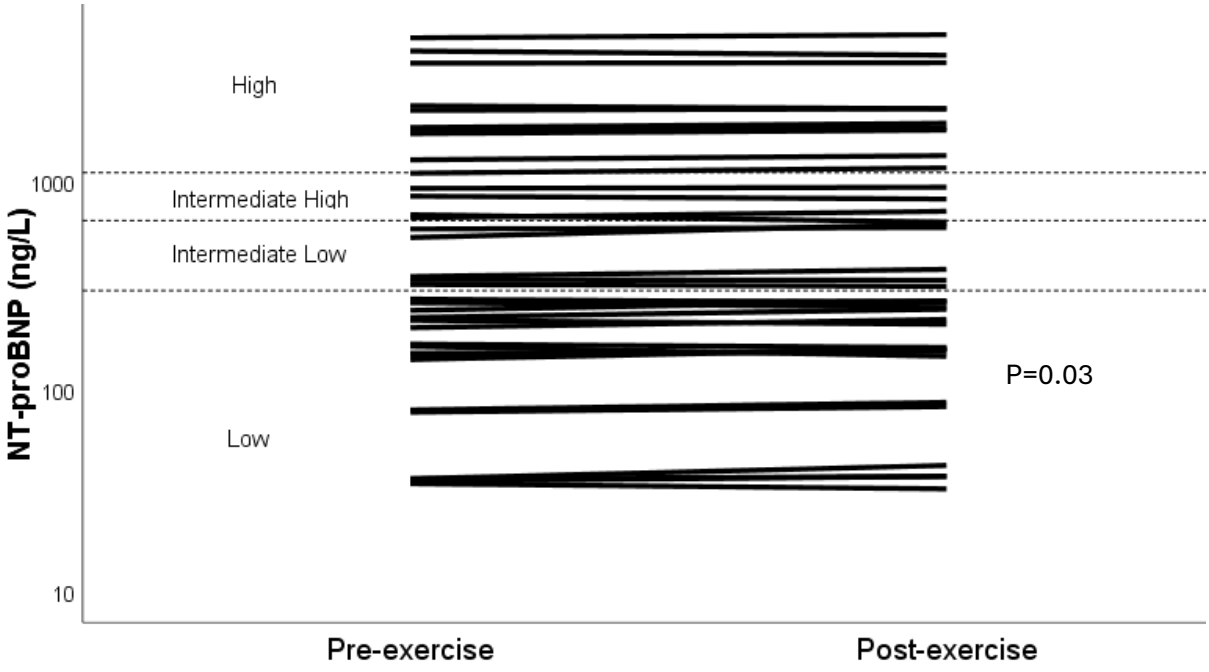
Exercise as an influencing factor on NT-proBNP

Thirty-seven patients performed an ISWT with pre- and post-exercise laboratory NT-proBNP and 36 patients who had pre- and post BNP. Paired analysis revealed a

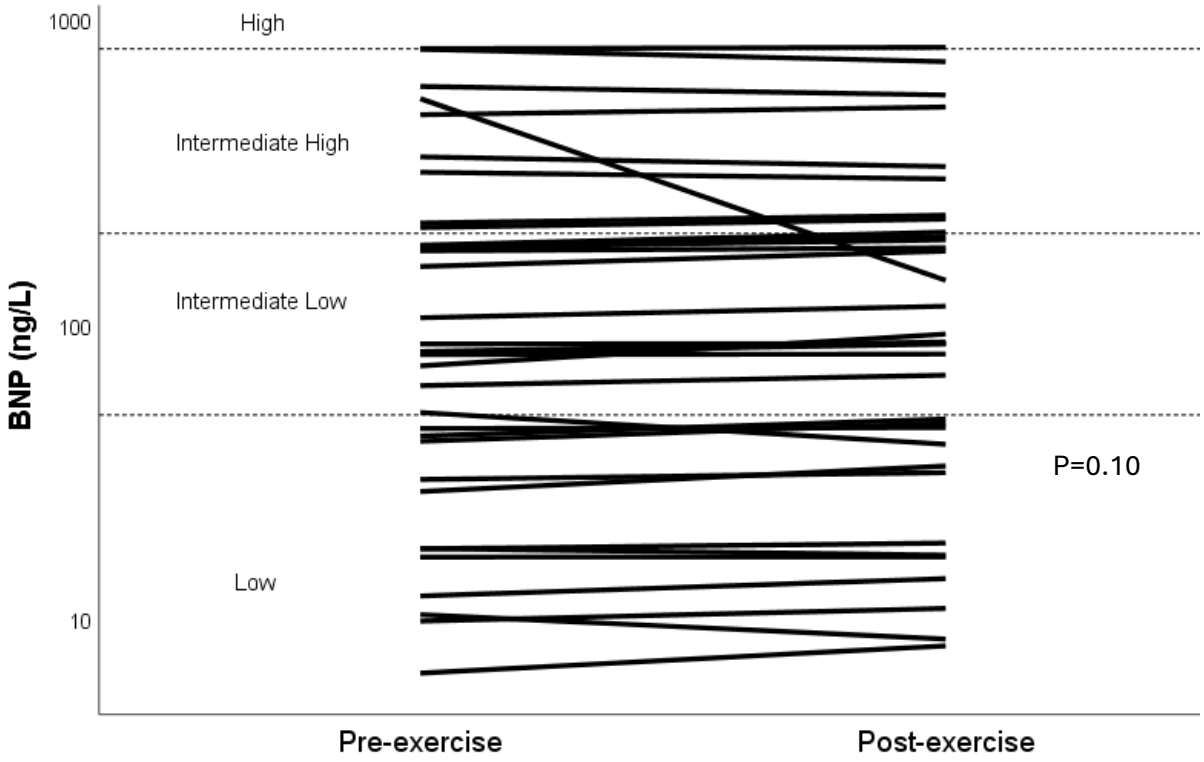
significant effect of exercise on NT-proBNP (p=0.031), however with a median difference of 7ng/L. The effect of exercise on BNP was not significant (p=0.1), with a median difference of 7 ng/L (Figure 11 A+B).

Figure 11 ***The effect of exercise on NT-proBNP and BNP***

A.



B.



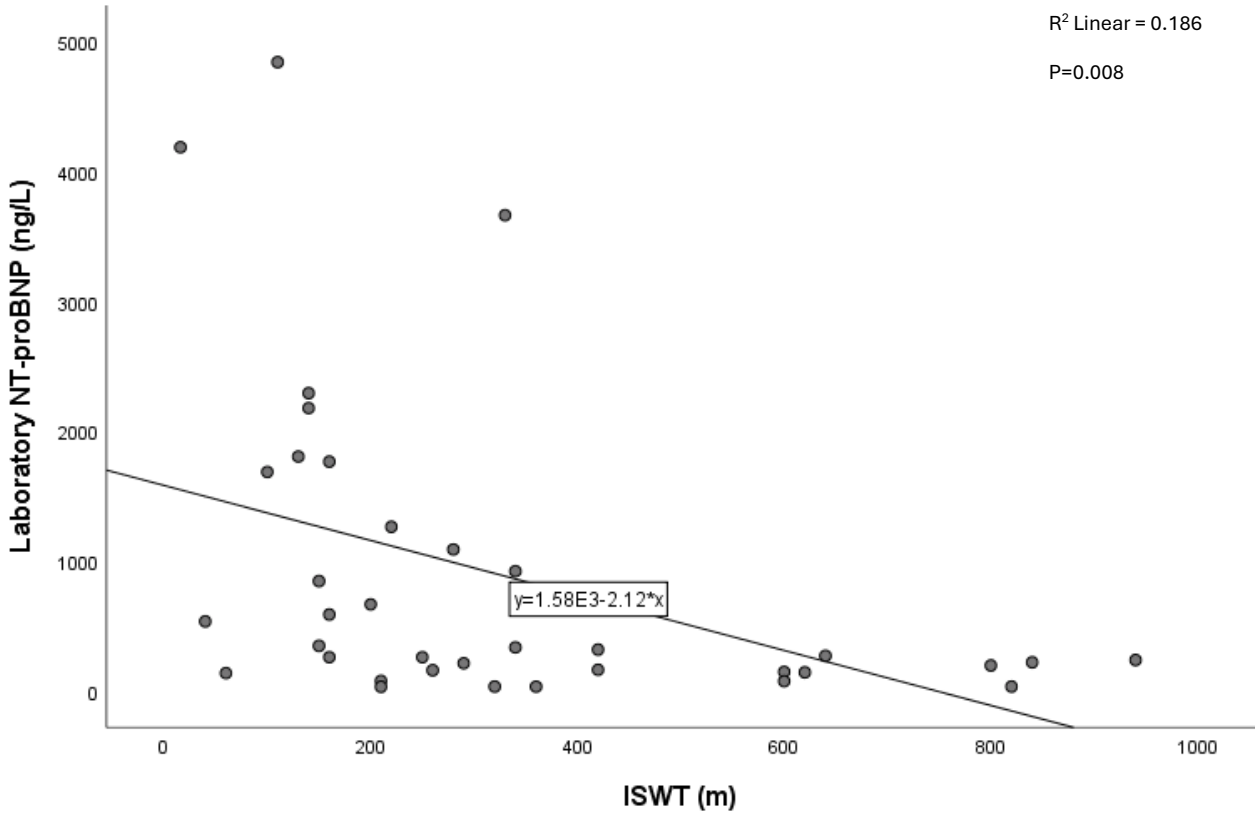
The effect of exercise on NT-proBNP (A) (n=37) and BNP (B) (n-36) pre and post incremental shuttle walk test. Dashed lines indicate thresholds for COMPERA 2.0 low risk, intermediate risk and high-risk status. Data analysed by Wilcoxon Signed Rank test.

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide

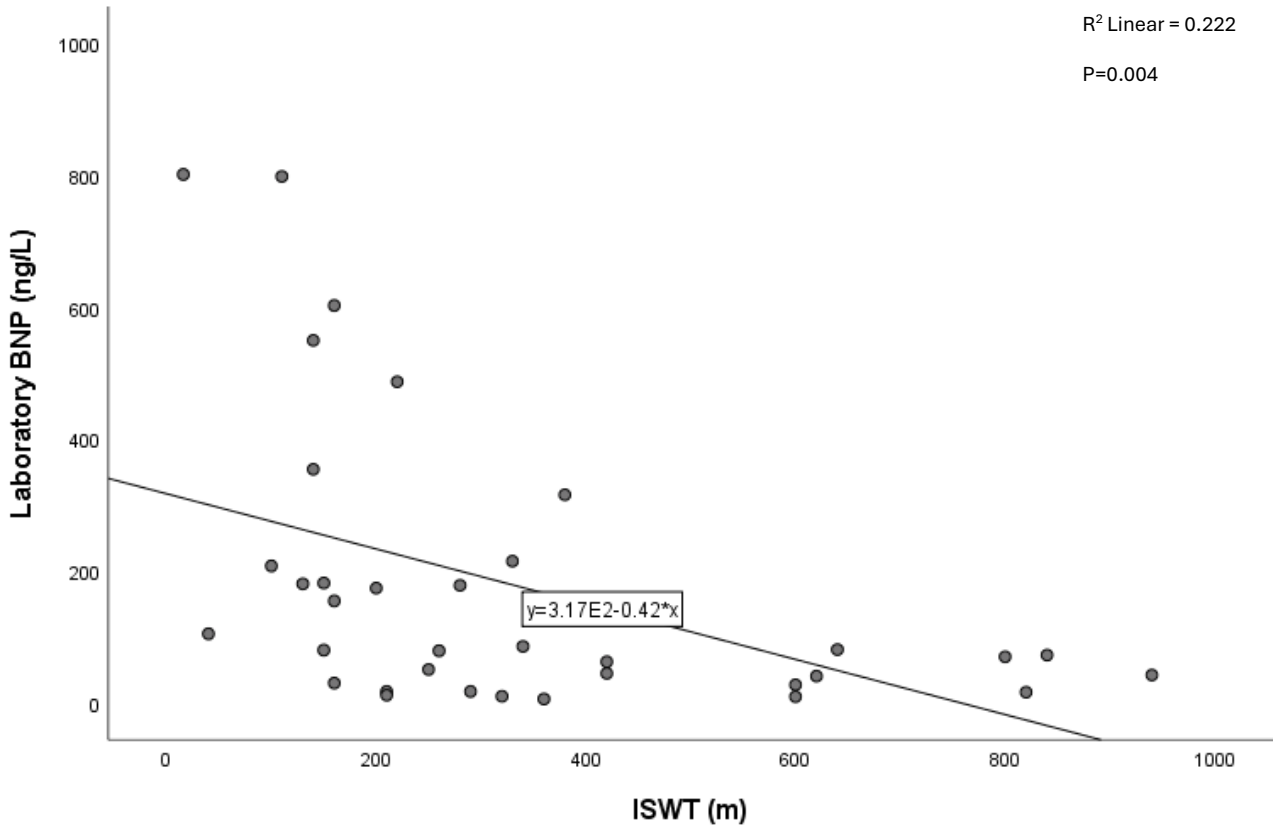
There was a weak linear relationship demonstrated between ISWT and baseline NT-proBNP (p=0.008) and baseline BNP (p=0.004) (Figure 12 A, B) and there was also no correlation between distance walked on ISWT and % change in NT-proBNP or BNP values following exercise (Figure 12 C, D).

Figure 12 ***The relationship between ISWT, NT-proBNP and BNP***

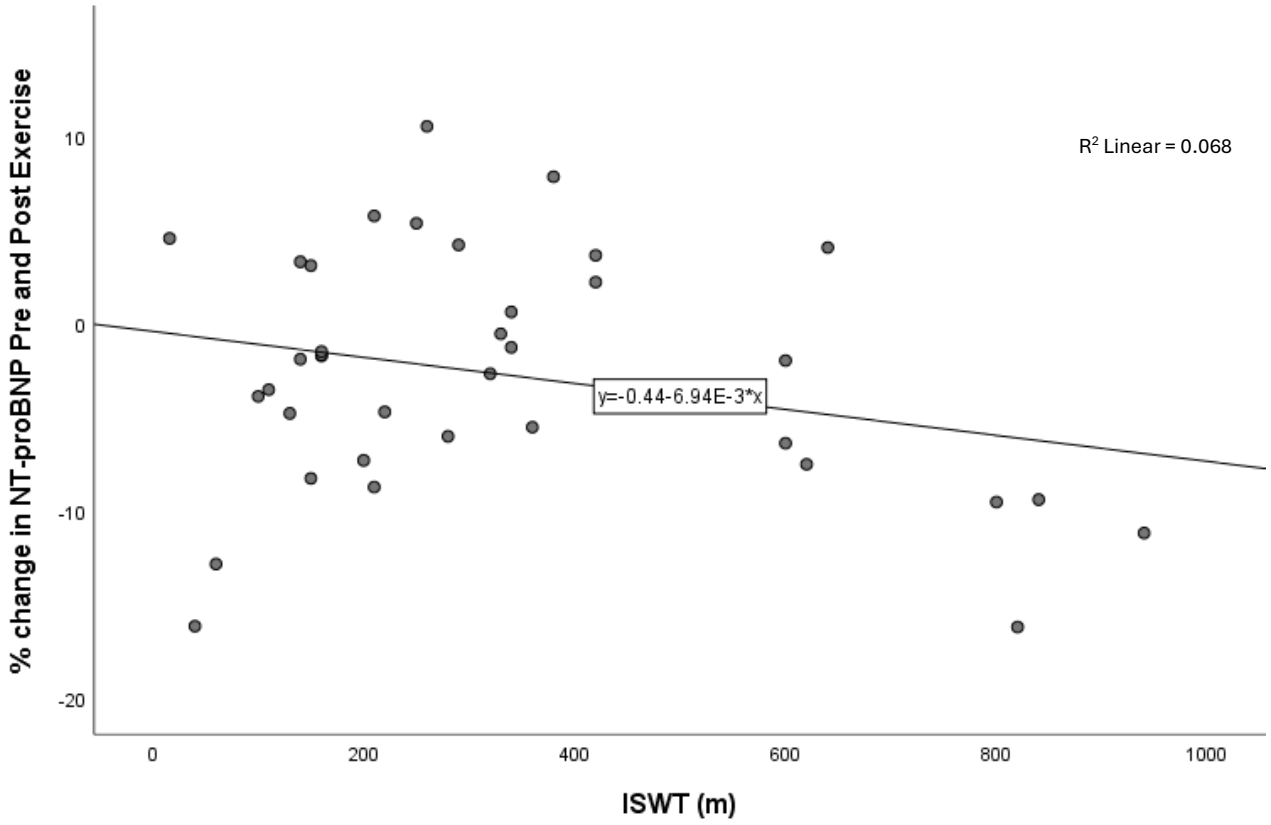
A.



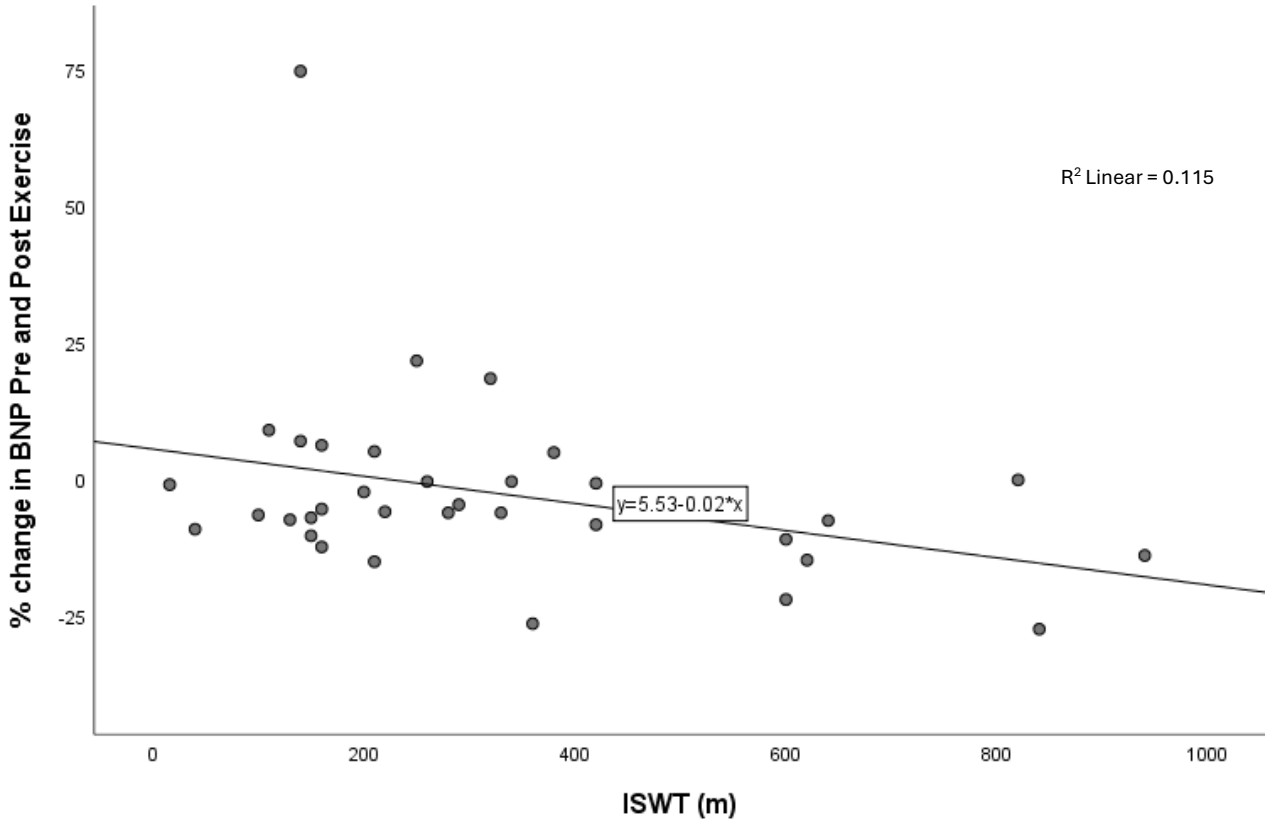
B.



C.


% change in NT-proBNP Pre and Post Exercise
10
0
-10
-20
0
200
400
600
800
1000
ISWT (m)
R² Linear = 0.068
y=-0.44-6.94E-3*x

D.


% change in BNP Pre and Post Exercise
75
50
25
0
-25
0
200
400
600
800
1000
ISWT (m)
R² Linear = 0.115
y=5.53-0.02*x

(A) Absolute laboratory NT-proBNP pre ISWT plotted against ISWT, (n37), (B) Absolute laboratory BNP pre ISWT plotted against ISWT, (n=36). (C) % change in laboratory NT-proBNP pre and post ISWT, (n=37), (D) % change in laboratory BNP, (n=36).

Abbreviations: BNP= brain natriuretic peptide, NT-proBNP= N-terminal prohormone of brain natriuretic peptide, ISWT= incremental shuttle walk test

Discussion

This study provides insights into the reliability of near-patient tests of NT-proBNP and BNP and demonstrates potential for NT-proBNP to be used to remotely monitor patients with PAH. POCT for both NT-proBNP and BNP correctly identified the patients' risk category making POCT a reliable tool in the setting of PAH. We also observed that a pre-processing time delay did not significantly alter NT-proBNP laboratory results for samples returned within 5 days of sampling.

Demographics

Over a 19-month period, 41 patients were enrolled, with a total of 56 study visits. The majority (83%) of patients recruited had a diagnosis of IPAH and female patients comprised 71% of the cohort, in keeping with registry data showing a female predominance in IPAH. [279] Reflecting a real-world out-patient population, the patients had a variety of co-morbidities including a fifth of patients with minor lung disease.

Point of care NT-proBNP /BNP and Laboratory NT-proBNP/BNP

The first aim of this study was assessing POCT and laboratory measurements of NT-proBNP and BNP. To examine the relationship between laboratory and POCT NT-proBNP and BNP several statistical methods were utilised. For NT-proBNP laboratory versus NT-proBNP POCT Bland-Altman demonstrated wide limits of agreement, however, Passing-Bablok deemed the two assays to be equivalent. ICC was in the excellent range and Lin's CCC showed substantial concordance. When examined against the COMPERA 2.0 4-strata risk tool, 94% of POCT results were in the correct risk grouping according to their laboratory comparator.

For BNP laboratory versus BNP POCT, Bland- Altman analysis showed that limits of agreement were wide and the Passing-Bablok was not equivalent. ICC was high, however Lin's CCC demonstrated moderate concordance. Although less than NT-proBNP, when examining BNP POCT performance against the COMPERA 2.0 4-strata risk tool, 86% of POCT results were correctly identified patients into the correct risk group. Therefore, in practical terms, both NT-proBNP and BNP POCT results were able to identify risk thresholds.

The major benefit for utilising NT-proBNP and BNP POCT in clinical practice is the much shorter processing time compared to formal laboratory analysis. Processing time for laboratory NT-proBNP and BNP will be dependent upon the laboratory, and in our experience the result is usually available the following day. In contrast for POCT testing for NT-proBNP and BNP, results are available in approximately 25 minutes. Laboratory testing of NT-proBNP and BNP is complex, requires specialist equipment and trained laboratory staff. [195] Multiple studies have explored the ease of processing POCT. In one study, after 2 training sessions (1.5 h), GPs were successfully taught to use and interpret BNP POCT in the setting of left heart failure [196] and another "untrained user study" using POCT BNP only gave standard user instructions to operators and reported comparable values to clinicians experienced with the same device. [197] BNP POCT assays are already established in the screening of patients presenting with unexplained breathlessness and screening for heart failure. [192]

An important consideration is cost of NT-proBNP and BNP POCT in comparison to NT-proBNP and BNP laboratory processing. The National Institute for Health and care Excellence (NICE) estimates the cost of laboratory NT-proBNP/BNP to be £15-25. In comparison it costs approximately £29 for the testing kit needed per test to process

each POCT NT-proBNP/BNP. Other costs such as the initial up-front and maintenance cost for the processing machines and the staff time for training and processing need to be considered for both laboratory and POCT.

Remote monitoring

Remote monitoring of liver function tests for patients who are on endothelin receptor antagonists, is used in the setting of PH. [280] These patients are sent a pre-labelled box containing all the equipment needed and are asked to attend their local phlebotomy service. [281] To our knowledge remote monitoring for laboratory NT-proBNP has not been previously explored. In our analysis we found that agreement was similar between the delayed postal laboratory samples in comparison to the immediately processed laboratory samples. Eighty-eight percent of samples were returned to the laboratory and processed without issue. It was noted there were two anomalous values, one returned at 2 days and the other 7 days. We did note increasing variability of results with increasing time delay (Figure 10). This was not statistically significant but highlights that longer delays in analysis could potentially impact on results. This study did not attempt to control for factors such as time of processing or temperature to assess remote monitoring of NT-proBNP in a real-world setting. However, we did assess the effect of exercise prior to sampling.

Effects of exercise on NT-proBNP and BNP

There are limited data on exercise and its impact on NT-proBNP and BNP results. There are convincing data from pooled systematic review of 27 studies to suggest that in patients with heart failure, rehabilitation exercise programmes comprising several sessions over a sustained period lowers NT-proBNP and BNP levels, implying

improved heart function. [282] However there are limited data examining shorter burst of exercise in patients with heart failure or PAH. A study examining BNP in 13 patients with stable heart failure, patients either performed an endurance cycle for 30 minutes or a high intensity training (HIT) session. BNP was increased in both types of exercise, but more so immediately following the HIT session and fell at 2 hours to baseline. For the endurance cycle there was a rise immediately following cessation of exercise, but BNP continued to rise 2-hours following cessation of exercise. [283] A further study examining 20 patients with PAH who had NT-proBNP measured at different time points, demonstrated a rise in all but one patient at maximum exercise during ergospirometry testing (baseline NT-proBNP 1278±998 pg/ml, peak exercise 1592±1219pg/ml) indicating NT-proBNP may have an immediate rise with exertion. [284] This was also examined in 63-therapy-naïve PAH patients, who demonstrated an increased in NT-proBNP at baseline to peak exercise (1414pg/ml at baseline to 1500pg/ml at peak exercise). [285] In our study we examined 37 patients who underwent an ISWT, which is regarded as a maximal test. Laboratory NT-proBNP and BNP were sampled prior to the ISWT and immediately after to provide a comparison. We found a significant rise in laboratory NT-proBNP (p=0.031); however, the median difference was only 7ng/L and unlikely to be clinically significant. In contrast no significant difference between BNP laboratory samples was noted. Our results suggest that exercise around the time of venous blood sampling is unlikely to increase NT-proBNP or BNP levels such that it would impact on decision making in PAH patients.

Limitations

This study was limited by its inclusion of only patients with PAH, however, it would be unlikely for samples from patients with other forms of PH, such as CTEPH, to alter assay performance. Although we observed no clinically significant change in NT-proBNP or BNP levels following exercise it is possible that more prolonged exercise may have impacted on levels. However, it suggests that day to day activities and standard field walking tests performed in the evaluation of patients with PH are unlikely to have a clinically significant impact on NT-proBNP and BNP levels. For POCT, a number of NT-proBNP POCT test kits failed the quality control process, due to temporary problems with kit temperature regulation, highlighting that POCT test kits are temperature sensitive, and care must be taken to maintain correct storage of equipment. Twelve percent of postal NT-proBNP samples were not processed because they were 'lost' in the post (the duration of this study encompassed the Royal Mail postal strikes; it is possible that this may have had an influence) or had been mistakenly discarded and tracking of returned samples would be important in the clinical application of remote monitoring.

In conclusion

For both NT-proBNP and BNP, POCT can provide an alternative to laboratory testing and has the added value of being quick and easy to process and available at the time of the clinical consultation. Postal laboratory NT-proBNP samples provide reliable results highlighting that NT-proBNP could be incorporated into remote clinical assessments.

Chapter 4: Systematic pulmonary embolism follow-up increases diagnostic rates of chronic thromboembolic pulmonary hypertension and identifies less severe disease: results from the ASPIRE Registry

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Take home message

Systematic follow-up after acute PE at 3 months, increases population-based rates of both CTEPH diagnosis and pulmonary endarterectomy, identifying patients earlier with less severe disease. The absence of major transient risk factors for pulmonary embolism and CT features at diagnosis predict the development of CTEPH.

Abstract

Background

Diagnostic rates and risk factors for the subsequent development of CTEPH following PE are not well defined.

Methods

Over a 10-year period (2010-2020), consecutive patients attending a PE follow-up clinic in Sheffield (population 554,600) and all patients diagnosed with CTEPH, at a PH referral centre in Sheffield (referral population estimated 15-20 million) were included.

Results

Of 1956 patients attending the Sheffield PE clinic 3 months following a diagnosis of acute PE, 41 were diagnosed with CTEPH with a cumulative incidence of 2.10%, with 1.89% diagnosed within 2 years. Of 809 patients presenting with pulmonary hypertension and diagnosed with CTEPH, 32 were Sheffield residents and 777 non-Sheffield residents. Patients diagnosed with CTEPH at the PE follow-up clinic had shorter symptom duration ($p<0.01$), better exercise capacity ($p<0.05$) and less severe pulmonary haemodynamics ($p<0.01$), compared to patients referred with suspected PH. Patients with no major transient risk factors present at the time of acute PE had a significantly higher risk of CTEPH compared to patients with major transient risk factors, OR of 3.6 (95% CI 1.11 to 11.91, $p=0.03$), The presence of 3 CT features of PH in combination with ≥2 of 4 features of CTEPD at the index PE was present in 19% of patients who developed CTEPH and in 0% of patients who did not. Diagnostic

rates and PEA rates were higher at 13.2/million/year and 3.6/million/year for Sheffield residents compared to 3.9–5.2/million/year and 1.7–2.3/million/year for non-Sheffield residents, respectively.

Conclusions

In the real world setting a dedicated PE follow-up pathway identifies patients with less severe CTEPH and increases population-based CTEPH diagnostic and pulmonary endarterectomy rates. At the time of acute PE diagnosis the absence of major transient risk factors, CT features of PH and chronic thromboembolism are risk factors for a subsequent diagnosis of CTEPH.

Introduction

PE is a condition in which thrombus, usually embolised from the veins of the pelvis or lower limbs, obstructs the pulmonary arterial vascular bed. The incidence of PE is estimated at 60-70 per 100,000 per year [1] with a 1-year mortality of 15%. [207, 287] In survivors, patency of the pulmonary vasculature is restored, in most patients, within the first few months. [209] However, PE may not resolve and patients may also develop a chronic obstructing microvasculopathy. [288, 289] This can lead to elevation of pulmonary artery pressure and if untreated, the development of progressive right heart failure. [113] Once a life-limiting condition, the management of CTEPH, has been transformed by the development of a multimodal treatment approach including PEA, BPA and pulmonary vasodilator therapy. [61, 144, 290]

Consequently, there is interest in strategies to diagnose CTEPH. [61, 93, 210, 291] Published data notes an incidence of CTEPH following acute PE of 0.1-9.1% [231, 292–297], reflecting differences in cohorts studied, study design and the tools used to establish the diagnosis of CTEPH. In a meta-analysis the incidence of CTEPH following PE was estimated at 0.56% in 2 studies of “all comers” and in another cohort that included only survivors the incidence was 3.21% and 2.78% in survivors without major comorbidities. [298] Recently, a large prospective, multicentre, observational cohort study, enrolled 1017 patients with acute PE and followed these patients with a standardised assessment at 3,12 and 24 months including a clinical assessment, natriuretic peptides, exercise testing and echocardiography. In that study CTEPH was diagnosed in 16 (1.6%) of patients with an estimated 2-year cumulative incidence of 2.3%. [228] If one were to extrapolate from the incidence of acute PE one would expect rates of CTEPH diagnosis of up to 15 cases per million/year. [206] However, observed

rates of CTEPH diagnosis from data reported in literature from the UK and other European countries show observed rates of CTEPH diagnosis to be 4-7 cases per million/year [232] and PEA rates of 0.9-1.7 per million/year. [299] Consequently, there has been interest in developing strategies to increase diagnostic rates for CTEPH and to consider early detection approaches in at risk populations. [300, 301] ESC/ERS guidelines on PE [210] recommend that patients should be systematically evaluated following acute PE to assess for CTEPH. However, there is no consensus on how patients should be followed-up after an episode of acute PE. Algorithms for predicting CTEPH [234] or ruling out CTEPH [235] have been proposed but have not been widely incorporated into clinical practice.

The primary aim of this study was to assess the impact of an integrated acute PE pathway, in a large volume tertiary referral centre with expertise in acute PE and the management of CTEPH and assess whether such a pathway can achieve high CTEPH diagnostic rates in a real-world setting.

Methods

Consecutive patients who attended a dedicated follow-up clinic after an acute episode of PE at Sheffield Teaching Hospitals NHS Foundation Trust and consecutive patients from the ASPIRE Registry diagnosed with CTEPH at the Sheffield Pulmonary Vascular Disease Unit between March 2010 and March 2020 were included. The ASPIRE Registry includes data on consecutive patients undergoing investigation at a PH referral centre based in Sheffield, UK from 2001 onwards. [278] Data collected as part of routine clinical care is stored within hospital clinical systems is then exported and anonymised, prior to analysis. During assessment patients undergo systematic evaluation including multimodality imaging and RHC in accordance with annually audited national standards of care. [300]

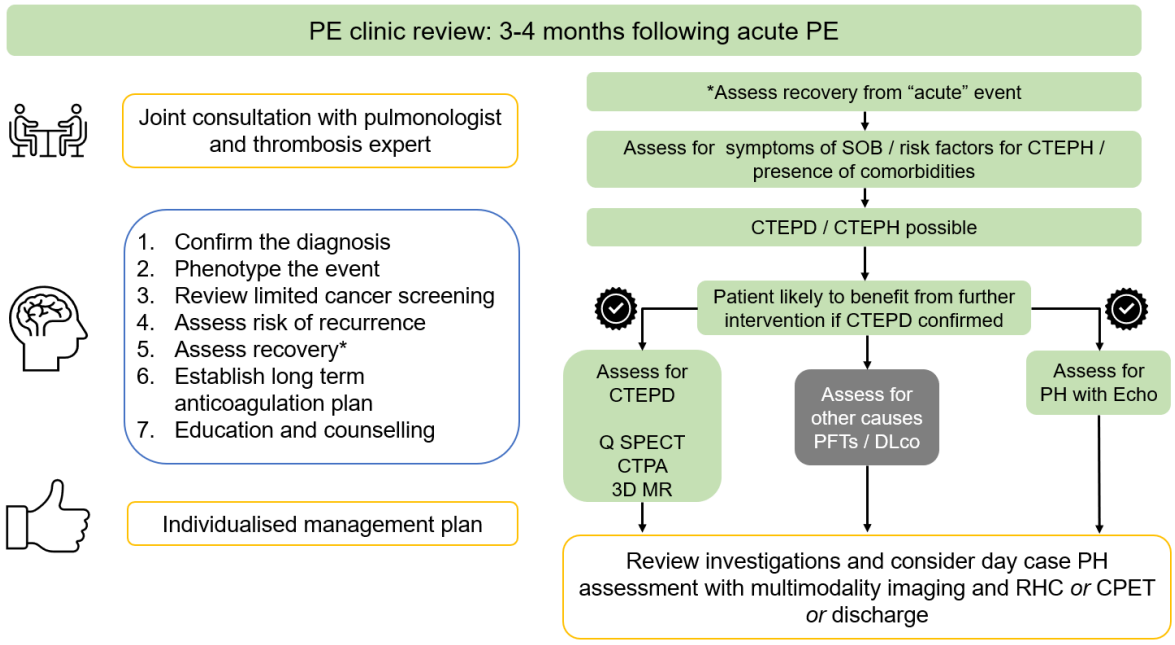
CTEPH was defined according to the recommendations of 2022 ESC/ERS PH guidelines [61] and required supportive imaging, a mPAP >20 mmHg and PVR >2 Wood units at RHC, with other causes of PH excluded. In occasional cases RHC data was not available, for example in patients who declined or where co-morbidities made invasive investigations inappropriate. In these cases, a diagnosis of CTEPH was made by multimodality imaging and expert opinion.

The Sheffield PE Service and PE follow-up clinic

From 2010 onwards, physicians managing Sheffield residents discharged with a new diagnosis of acute PE, were encouraged to refer patients for follow-up in the PE service, with the exception of patients with malignancy (who were managed in a separate clinical pathway) and patients who were deemed by the referring physician to be frail, where decisions with respect to anticoagulation were managed by the

thrombosis team. Patients were assessed within 1 week of discharge in an anticoagulation specialist nurse led clinic which included a clinical assessment and malignancy screening. If concerns were identified patients were discussed at a thrombosis MDT. Otherwise, patients were reviewed 3-4 months after their acute PE in a joint clinic, with a pulmonologist specialising in PE and a haematologist specialising in thrombosis. Patients were evaluated with respect to risk of VTE recurrence, duration of anticoagulation and assessed for possible CTEPH (Figure 13). CTEPH screening and early detection included a review of the clinical presentation and diagnostic imaging, assessment of symptoms and review of risk factors for CTEPH. In selected cases, further investigation for CTEPH including an assessment of lung perfusion and assessment of pulmonary artery pressure (Figure 13) was performed. Following this initial assessment selected patients with suspected PH were referred to the Sheffield PH referral centre where they underwent systematic evaluation with multimodality imaging (lung scintigraphy, CT pulmonary angiography, CMR with MR angiography and where there remained diagnostic doubt digital subtraction angiography) and RHC. Patients who were recovered from their acute PE or investigated and found not to have CTEPH were discharged with advice to represent for assessment if they developed symptoms of CTEPH. Unless the patient declines, all cases of CTEPH are referred to the UK national referral centre for PEA and BPA (Royal Papworth Hospital). [146] In order to establish an estimate of the number of patients diagnosed with acute PE in the Sheffield area during the duration of the study, all CTPA reports from the Sheffield 3D lab were evaluated from March 2010 to March 2020 and patients with a diagnosis of PE from a Sheffield post-code were identified.

Figure 13 ***Acute PE follow-up pathway and assessment for suspected CTEPH***



Abbreviations: PE= pulmonary embolism, SOB= shortness of breath, CTEPH= chronic thromboembolic pulmonary hypertension, CTEPD= chronic thromboembolic pulmonary disease, Q SPECT= Perfusion single-photon emission computerised tomography, CTPA= computerised tomography pulmonary angiogram, 3D MR= 3 dimensional magnetic resonance imaging, PFT= pulmonary function tests, DLco, diffusing capacity of the lungs for carbon monoxide, Echo= echocardiogram, RHC= right heart catheterisation, CPET= cardiopulmonary exercise testing

Note patients also receive a follow-up appointment with a thrombosis nurse specialist within the first week of discharge. To assess for CTEPD patients underwent Q SPECT or CTPA or less frequently 3DMR imaging.

Features of CTEPH on CTPA performed at the time of the index PE in Sheffield PE patients diagnosed with CTEPH at follow-up

For patients diagnosed with CTEPH attending the Sheffield PE clinic, CTPAs were retrieved and reviewed by a consultant radiologist experienced in pulmonary vascular disease. The presence or absence of CT features predictive of the presence of PH and CTEPD were recorded. Where all 3 features of PH were present; PA size ≥30mm, right ventricular outflow hypertrophy ≥6mm and right ventricular:left ventricular ratio ≥1

and at least 2 of 4 features of CTEPD (dilated bronchial arteries, arterial webs or bands, attenuated or occluded vessels and mosaic parenchymal perfusion pattern) were present, the patient was defined as having CTEPH at the time of the initial presentation. A control group of randomly selected patients who were not diagnosed during follow-up with CTEPH were also examined for features of PH and CTEPD at the time of the index PE.

Patient Groupings

All patients diagnosed with CTEPH during the study period were divided into 3 groups according to the origin of referral: Sheffield PE clinic, Sheffield residents referred with suspected PH, and non- Sheffield residents referred with suspected PH. Sheffield residents were defined as those who lived in a postcode area of the City of Sheffield. For the 3 patient groups, demographic, investigation and survival data were retrieved from the ASPIRE registry. [278]

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics v26 (SPSS, Chicago, IL, USA). Continuous variables were described by mean ± SD and data that was not normally distributed are shown as median ± IQR. Kruskal-Wallis statistical test with Bonferroni correction was used to compare the 3 groups. Event (death)- free survival from the date of diagnosis was estimated using the Kaplan-Meier method with comparison between groups performed by the log-rank test. A p-value <0.05 was deemed statistically significant.

Population data

Rates for annual incidence were based on a mean population of the city of Sheffield, derived from the 2011 and 2021 department of national statistics census data, of 554,600. The Sheffield PH referral centre is part of a UK national network, adhering to annually audited and published standards of care, and covers a referral population of 15–20 million. Ethical approval for this study was obtained through the ASPIRE registry (REC 22/EE/0011).

Results

Population

Between March 2010 and March 2020, 1956 patients attended the PE clinic and 850 patients from the ASPIRE Registry were diagnosed with CTEPH, 41 from the Sheffield PE clinic and 809 referred with suspected pulmonary hypertension. PE clinic patients were followed up within this study for a median of 66 (IQR 55) months. Demographic and baseline clinical characteristics of patients diagnosed with CTEPH, including those undergoing PEA, are shown in Table 10. Sheffield patients with CTEPH diagnosed at the PE clinic were older with a better exercise capacity than patients referred with PH (Tables 10 and 11).

Table 10 ***Baseline characteristics of patients diagnosed with chronic CTEPH and undergoing PEA***

Demographic Data	**Sheffield ***		**Non-Sheffield §**
	PE clinic	**Referred with suspected PH⁺**	**Referred with suspected PH**
All patients			
CTEPH (n)	41	32	777
Age at diagnosis (Years)	69.46 ± 14.49¥	63.38 ± 12.73	63.44 ± 4.95
Sex: Female (%)	22 (54)	10 (31)	382 (49)
WHO FC I/II/III/IV (%)	0/27/71/2	3/9/75/13	1/8/82/9
ISWT (m)	305 ± 218¥	221 ± 210	200 ± 188.30
mRAP (mmHg)	7.4 ± 3.9¥	10 ± 5.1	11 ± 6.0
mPAP (mmHg)	37 ± 10.1¥	42 ± 13.9	45 ± 11.5
PAWP (mmHg)	12 ± 3.7	12 ± 3.9	11 ± 6.09
CI (L/min/m2)	2.87 ± 0.65¥	2.54 ± 0.81	2.40 ± 0.76
PVR (Wood Units)	4.6 ± 2.4#¥	8.1 ± 6.5	8.3 ± 4.6
SvO_2 (%)	59.4 ± 24 #¥	62.9 ± 7.6	56.3 ± 19.09
Patients undergoing PEA			
PEA (n)	11	9	344
Age at diagnosis (years)	67.36 ± 12.01#¥	56.5 ± 13.44	58.11 ± 14.6
Sex: Female (%)	8 (73)	4 (50)	153 (44)
WHO FC I/II/III/IV (%)	0/45/55/0	0/25/74/0	0/8/86/5
ISWT (m)	360 ± 182	267 ± 163.4	241 ± 187
mRAP (mmHg)	7 ± 2.7	12 ± 6.05	10 ± 6.6
mPAP (mmHg)	41 ± 10.4	47 ± 9.8	46 ± 11.5
PAWP (mmHg)	11 ± 3.6	10.4 ± 6.6	11.9 ± 5.23
CI (L/min/m2)	3.1 ± 0.7¥	2.44 ± 0.75	2.42 ± 0.66
PVR (Wood Units)	5.4± 2.4	10.0 ± 5.8	8.29 ± 4.6
SvO_2 (%)	70 ± 4.8#¥	58 ± 7.51	62.3 ± 8.79

Abbreviations: PE= pulmonary embolism, PH= pulmonary hypertension, CTEPH= chronic thromboembolic pulmonary hypertension, WHO FC= world health organisation functional class, ISWT= incremental shuttle walk test, mRAP= mean right atrial pressure, mPAP= mean pulmonary artery pressure, PAWP= pulmonary arterial wedge pressure, CI= cardiac index, PVR= pulmonary vascular resistance, PEA= pulmonary endarterectomy

*Sheffield patients were defined as a Sheffield resident according to their residing postcode being within the city of Sheffield as per Sheffield City council. [§] Non-Sheffield patients were those residing outside the postcodes classed as the City of Sheffield.

[¥] p values <0.05 Sheffield acute PE follow-up vs. Non-Sheffield, [#] p values <0.05 Sheffield acute PE follow-up vs. Sheffield referred with PH.

[+] out-patient referrals (n=26, 81%), in-patient hospital transfers (n=6, 19%).

Table 11 ***Baseline characteristics of Sheffield residents diagnosed with CTEPH from the PE clinic compared to those referred with suspected PH***

		Sheffield	
		PE clinic	**Referred with suspected PH**
CTEPH (n)		41	32
Age at diagnosis (Years)		69.46 ± 14.49	63.38 ± 12.73
Sex: Female (%)		22 (54)	10 (31)
WHO FC I/II/III/IV (%)		0/27/71/2	3/9/75/13
Risk factors for venous thromboembolism (%)	**History of venous thromboembolism**	41 (100)*	12 (39)[+]
	Thyroid disease	5 (12)	1 (3)
	Cancer or myeloproliferative disease	4 (10)	7 (22)
	Hormonal therapies	1 (2)	1 (3)
	Splenectomy	1 (2)	1 (3)
	Pregnancy or puerperium	0	0
Co-morbidities (%)	**Chronic heart failure or coronary heart disease or valvular disease**	12 (29)	5 (16)
	Systemic hypertension	11 (27)	2 (6)
	Diabetes mellitus	8 (20)	3 (9)
	Atrial Fibrillation	5 (12)	2 (6)
	Chronic obstructive pulmonary disease	5 (12)	6 (19)
	Chronic renal failure	5 (12)	3 (9)
	Chronic liver disease	0	1 (3)
Symptom duration (%)	**<1 year**	76	38
	1-2 years	12	28
	>2 years	7	19
	Not clear	5	16
CTEPH operated (%)		11 (27)	9 (28)
CTEPH not operated (%)	**Disease distribution**	2 (5)	2 (6)
	Co-morbidities	6 (15)	8 (25)
	Mild disease	4 (10)	5 (16)
	Patient choice	16 (39)	5 (16)
	Not clear	1 (2)	1 (3)
	Died	1 (2)	2 (6)

PE=pulmonary embolism, PH= pulmonary hypertension, CTEPH= chronic thromboembolic pulmonary hypertension, WHO FC= world health organisation functional class

*14 (34%) of the patients diagnosed with CTEPH from the acute PE follow-up clinic had a previous history of VTE prior to their diagnosis with acute PE; [+]11 patients (2 after 2015) presented with a PE between 2010 and 2020 and were not referred to the PE Clinic but were subsequently referred directly to the PH referral centre from hospital out-patient clinics (n=9), as a hospital in-patient transfer (n=1) and from primary care (n=1).

Patients diagnosed with acute PE in Sheffield following CTPA

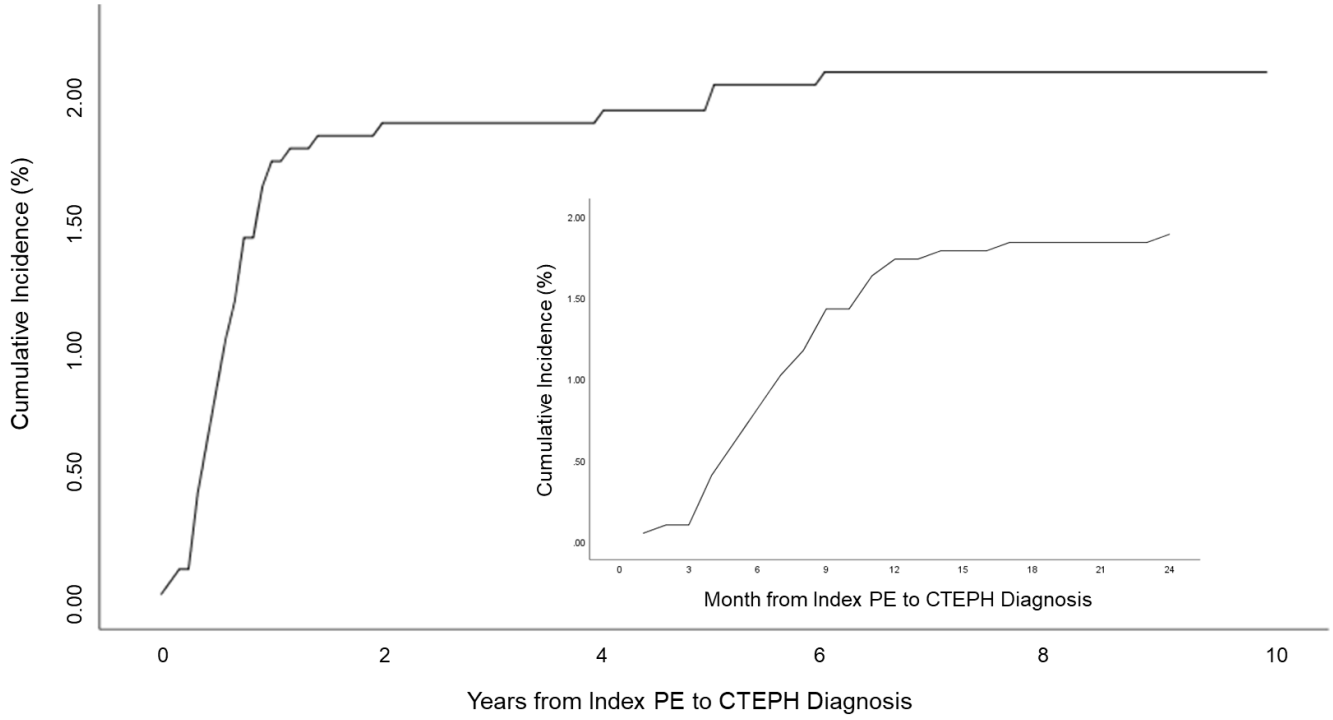
During the conduct of this study 20,494 patients from Sheffield underwent CTPA, 3289 patients (16%) were diagnosed with PE and 1956 (59%) of these patients were seen in the PE clinic.

Sheffield acute PE follow-up clinic

The 1956 patients reviewed in the PE clinic were seen at a mean of 3.95±1.94 months following a diagnosis of acute PE and where CTEPH suspected were investigated as per Figure 13. Between 2010-2020, 120 patients seen in the PE clinic were evaluated at the Sheffield PH referral centre. Of these, 41 were diagnosed with CTEPH, 7 PH associated with respiratory disease, 6 PH-LHD, 2 PAH (1 congenital heart disease, 1 associated with connective tissue disease), 62 had no PH (39 of these patients were classified as CTEPD non-invasively and 15 of these patients were diagnosed with CTEPD with a RHC) and in 2 patients no final diagnosis was possible.

The cumulative incidence of developing CTEPH from an index PE over the 10-year study period was 2.1%. CTEPH diagnosis was confirmed in 51% within 4 months, 71% within 6 months, 88% within 12 months and 90% within 24 months of first PE clinic appointment. The cumulative incidence of CTEPH diagnosis from the incident PE event is shown in Figure 14. The estimated 2-year cumulative incidence from the index PE event was 1.89%. Over the 10-year period a single patient was discharged with a non-invasive diagnosis of CTEPD but subsequently represented and was diagnosed with modest haemodynamic CTEPH 4.3 years later.

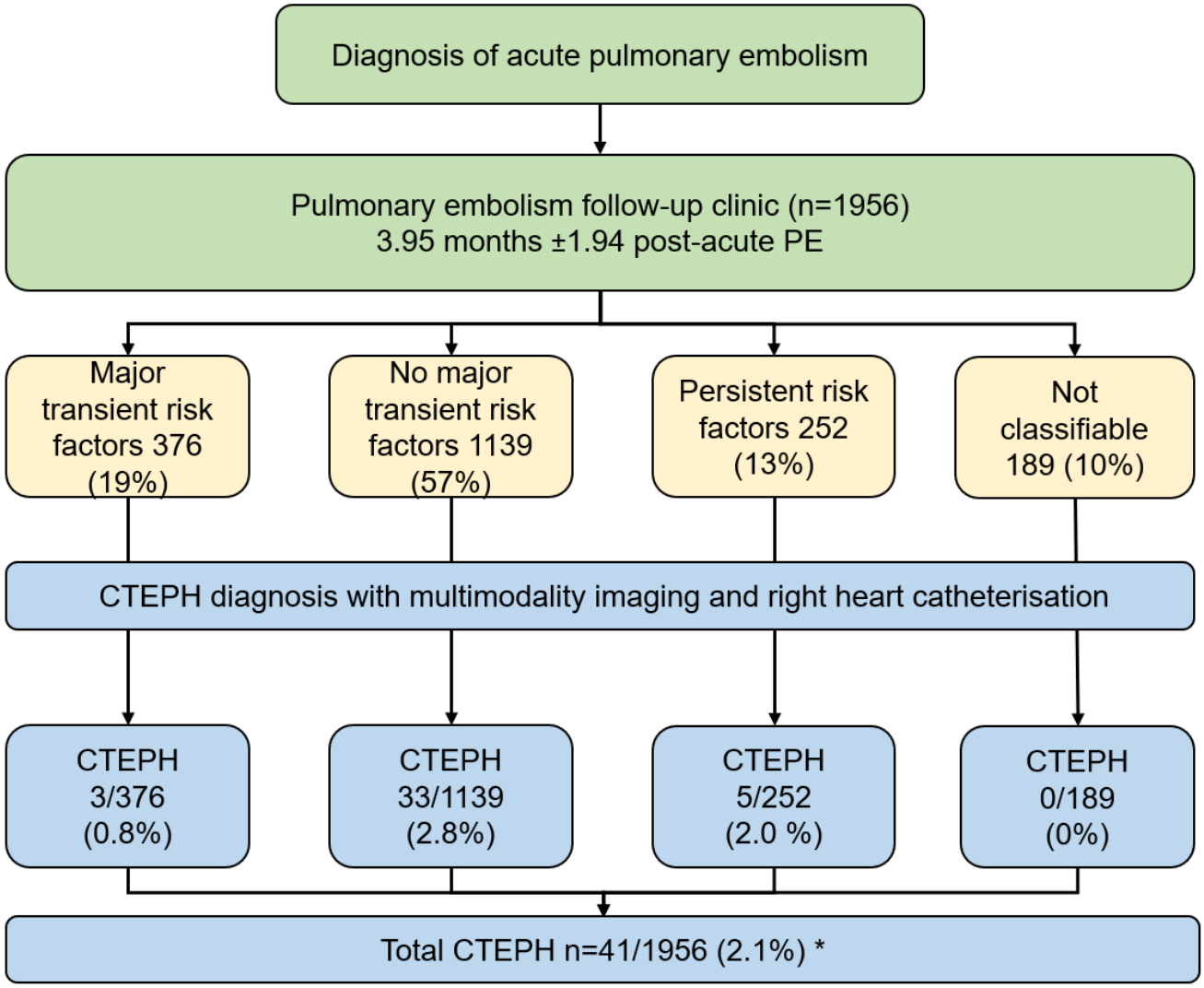
Figure 14 ***Total and 2-year cumulative incidence of CTEPH from index PE event (n=1956)***



Abbreviations: PE=pulmonary embolism, CTEPH=chronic thromboembolic pulmonary hypertension

Patients with no major transient risk factors present at the time of acute PE had a significantly higher risk of CTEPH compared to patients with major transient risk factors, OR of 3.6 (95% CI 1.11 to 11.91, p=0.03), however, there was no significant difference between patients with persistent risk factors and those with major transient risk factors OR of 2.49 (95% CI 0.59-11.50, p=0.22), Figure 15.

Figure 15 ***Flow chart showing acute PE pathway and impact of risk factors for acute PE on diagnosis of CTEPH***

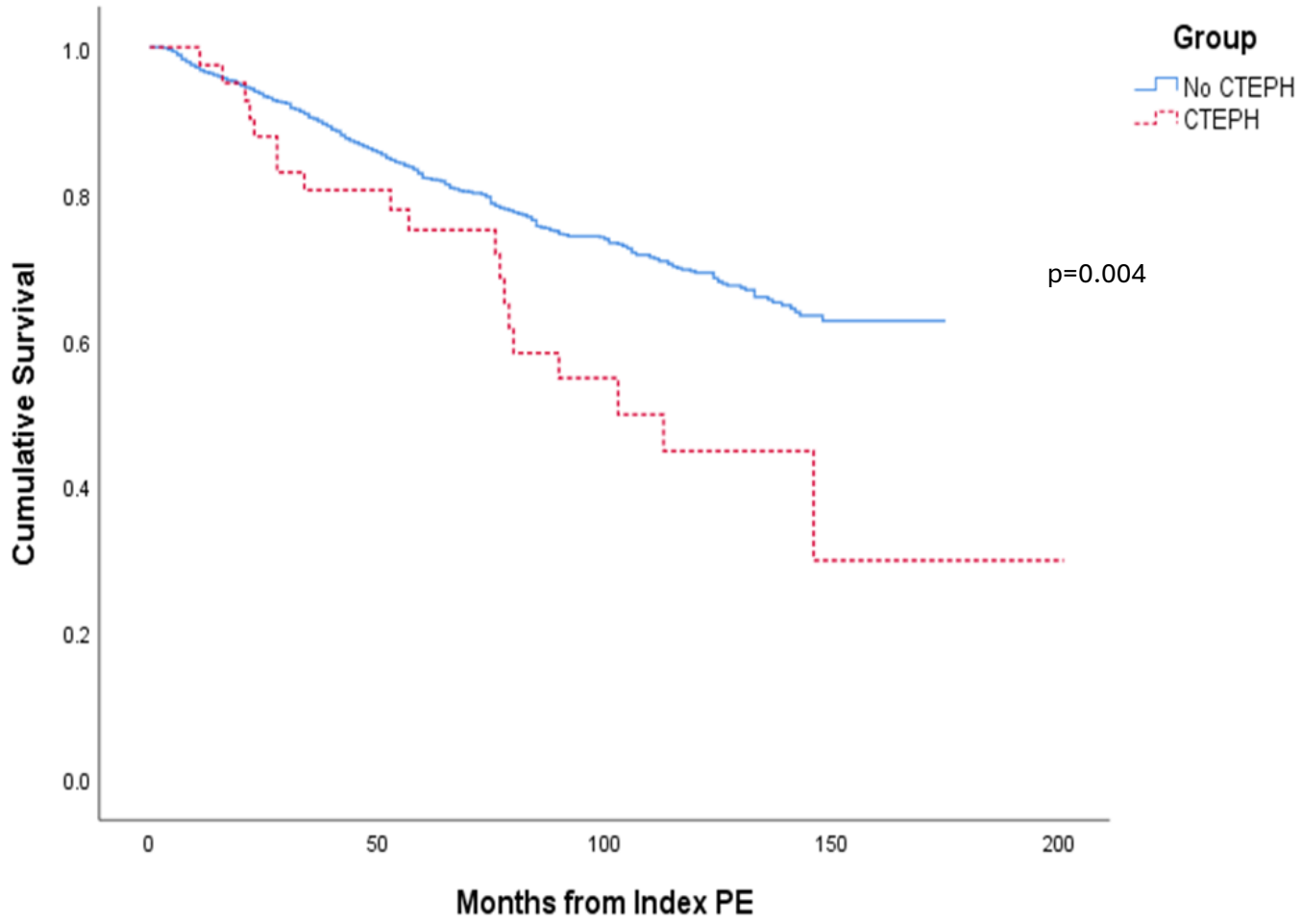


Abbreviations: PE= pulmonary embolism, CTEPH=chronic thromboembolic pulmonary hypertension
*All patients diagnosed at RHC apart from 2 patients who declined RHC, diagnosis in these cases was made based on multimodality imaging and expert opinion.

At the time of CTEPH diagnosis all patients had received at least 3 months of anticoagulation with 26 patients (63%) receiving warfarin, 13 (32%) a direct oral anticoagulant (DOAC) and 2 (5%) low molecular weight heparin. From 2015 onwards, 13 (54%) received a DOAC, 10 (42%) received warfarin and 1 (4%), low molecular weight heparin.

Patients from the PE clinic diagnosed with CTEPH had a significantly worse survival compared to patients not diagnosed with CTEPH; the 1, 3 and 5-year survival for patients attending the PE clinic with CTEPH versus those without CTEPH were 95% vs 96%, 78% vs 89% and 73% vs 83%, respectively, p=0.004, Figure 16. There was no significant difference in survival for patients undergoing PEA diagnosed in the PE clinic compared to those presenting with suspected PH with 1 and 3-year survival of 100% vs 95% and 100% vs 89%, respectively p=0.08.

Figure 16 ***Kaplan-Meier Survival curve for patients who attended the PE clinic split into those diagnosed or not diagnosed with CTEPH***



Numbers at Risk

▬	1901	1433	567	74	0
·····	41	30	12	1	1

Abbreviations: CTEPH= chronic thromboembolic pulmonary hypertension, PE= pulmonary embolism

Signs of pre-existing CTEPD and CTEPH on the CTPA at the time of the index PE in patients diagnosed with CTEPH from the Sheffield PE clinic

CTPAs from the index PE were available for 36 of 41 patients diagnosed with CTEPH from the Sheffield PE clinic and features of CTEPD and PH were compared to a control group of 36 patients from the Sheffield PE clinic not diagnosed with CTEPH during the follow-up period (Table 12). Two or more features of CTEPD and all 3 features of PH were present in 12 (33 %) and 13 (36%) of patients diagnosed with CTEPH compared to 1 (3%) and 2 (6%) of patients not diagnosed with CTEPH. Features defined as indicative of CTEPH at diagnosis (≥2 CT features of CTEPD and all 3 CT features of PH) were present in 7 (19%) of patients diagnosed with CTEPH compared to 0 (0%) of patients not diagnosed with CTEPH during follow-up. Figure 17 shows illustrative imaging from the time of the index PE event from a patient who went on to develop CTEPH during the study period compared to a patient who did not.

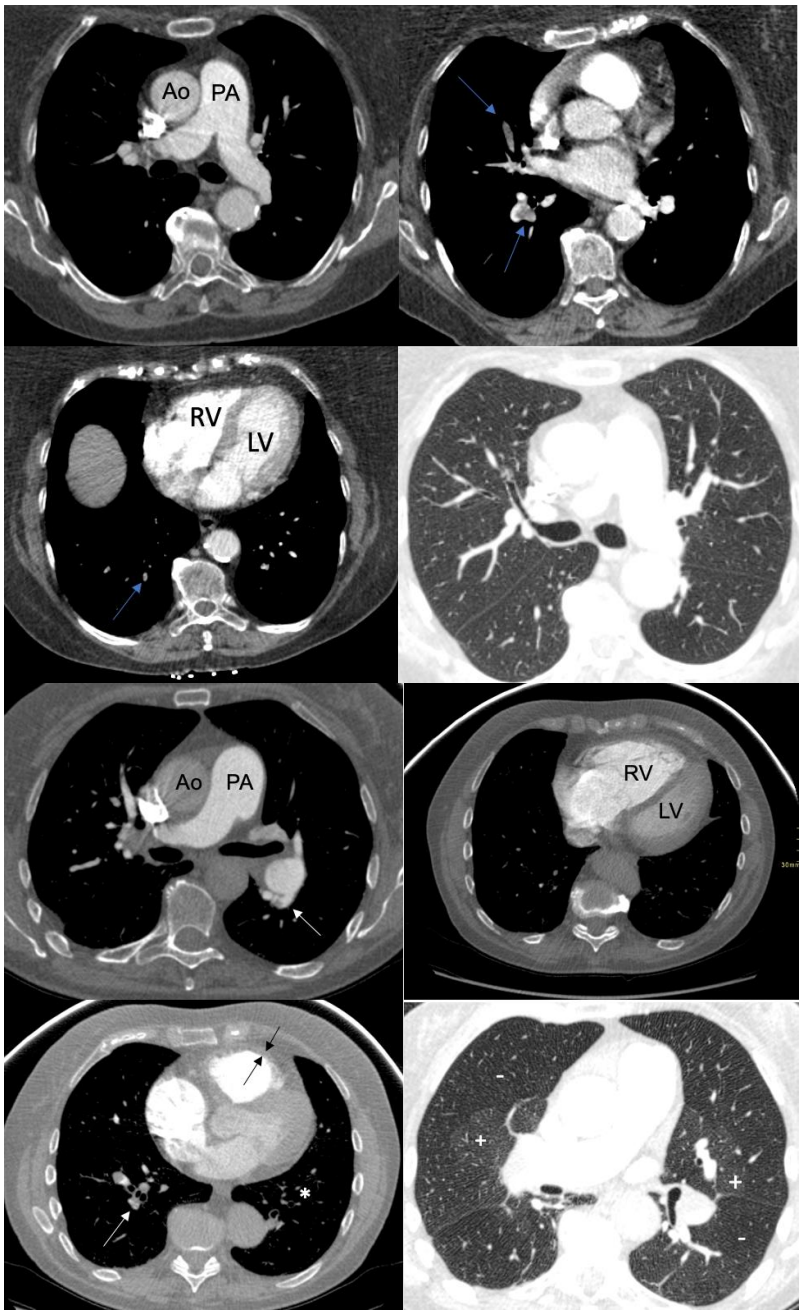
Table 12 **Features present on initial CTPA at time of PE diagnosis in patients with subsequent diagnosis of CTEPH and a randomly selected control group of patients not diagnosed with CTEPH**

	CTEPH +ve (n=36)	**CTEPH -ve (n=36)**
Demographics:		
Age, years (mean ± SD)	69.9 ± 13.9	68.5 ± 11.95
Sex M/F n, (%)	18 (50)/18 (50)	16 (44)/20(56)
Features suggestive of PH:		
PA ≥ 30mm n, (%)	24 (67)	11 (31)
RV:LV ≥1 n, (%)	28 (78)	13 (36)
RVOTH ≥6mm n, (%)	16 (44)	3 (8)
All 3 features suggestive of PH present n, (%)	13 (36)	2 (6)
Features suggestive of CTEPD:		
Dilated bronchial arteries, yes/no (%)*	10 (28)	2 (6)
Arterial webs or bands, yes/no (%)	4 (11)	1 (3)
Attenuated or Occluded vessels combined, n (%)	13 (36)	1 (3)
Mosaic parenchymal perfusion pattern yes/no n, (%)	8 (22)	2 (6)
2 or more features suggestive of CTEPD n, (%)	12 (33)	1 (3)
Features suggestive of CTEPH at index event:		
3 features of PH and ≥ 2 features of CTEPD n, (%)	7 (19)	0 (0)

Abbreviations: PA=pulmonary artery, RV:LV=right to left ventricular ratio, RVOTH=right ventricular outflow tract hypertrophy, PH=pulmonary hypertension, CTEPD=chronic thromboembolic pulmonary disease, CTEPH=chronic thromboembolic pulmonary hypertension

CTPA at the time of diagnosis of sufficient diagnostic quality were available for review in 36/41 of CTEPH +ve patients and are compared to a randomly selected control group of 36 patients not diagnosed as having CTEPH during the conduct of the study. PA pulmonary artery diameter; RV:LV ratio of internal right ventricular to left ventricular diameter; RVOTH thickness of right ventricular outflow tract. *assessment of bronchial artery size was not possible due to lack of contrast opacification in 9 (25%) of CTEPH +ve patients and 6 (17%) of CTEPH -ve patients

Figure 17 ***CTPAs from the Sheffield PE Clinic from the index PE in a patient who did not go on to develop CTEPH (Top) and a patient who went on to develop CTEPH (Bottom)***



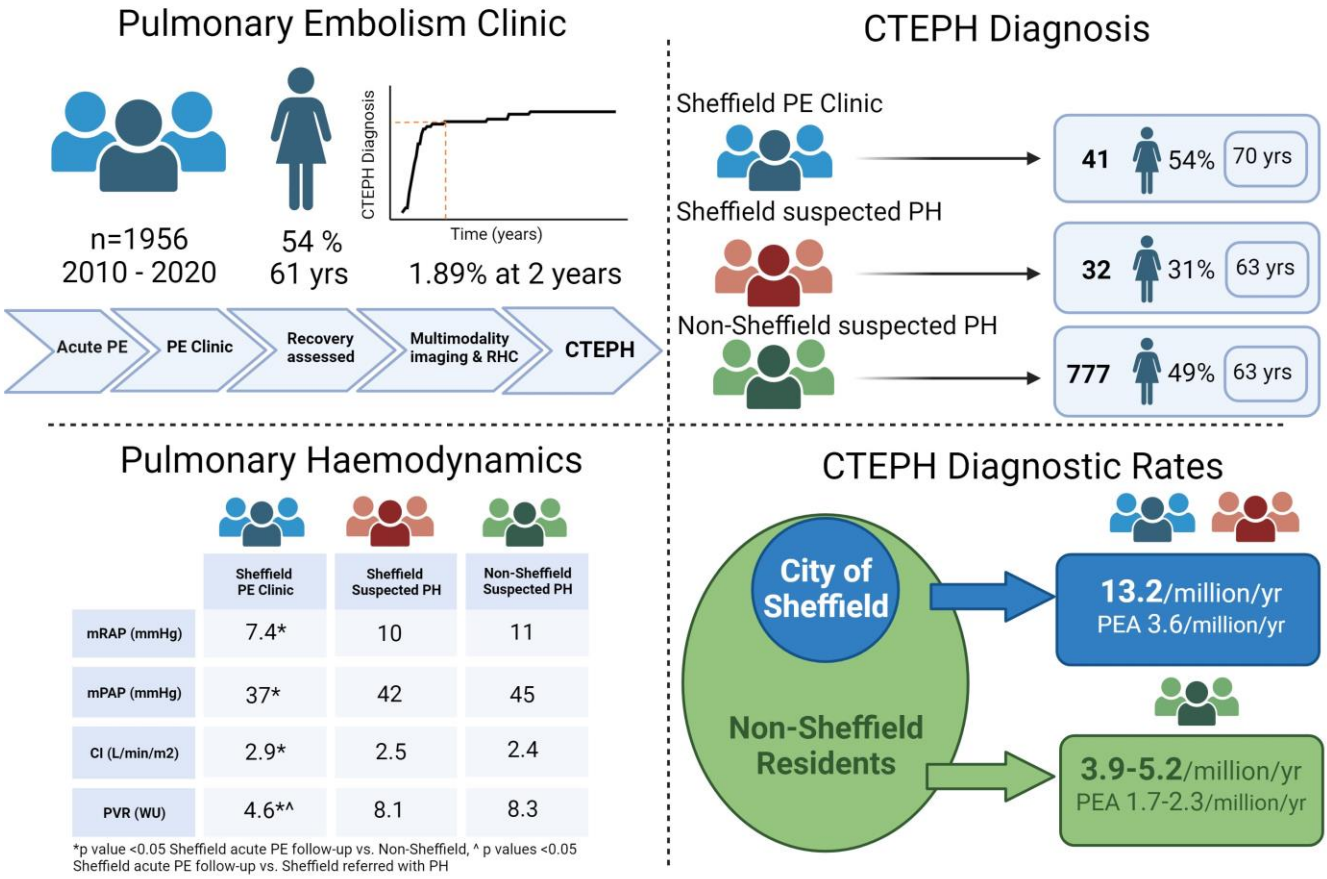
Top: of a patient from the time of the index PE who did not go on to develop CTEPH; note mild PA dilation, but a RV:LV ratio <1, no RVOTH and no features of CTEPD, filling defects shown by blue arrows. Figure 17 Bottom: of a patient from the time of the index PE who went on to develop CTEPH; note all 3 features of pulmonary hypertension: PA≥30mm, RVOTH≥6mm (between black arrows) and RV:LV≥1 and 3 features of CTEPD; arterial webs (white arrows) attenuated vessels (white asterix) and mosaic parenchymal perfusion pattern + and -).

Abbreviations: Ao= aorta, CTEPD= chronic thromboembolic pulmonary disease, CTEPH= chronic thromboembolic pulmonary hypertension, LV= left ventricle, PA= pulmonary artery, RV= right ventricle; RVOT= right ventricular outflow tract hypertrophy

Characteristics of patients diagnosed with CTEPH including those undergoing pulmonary endarterectomy

A total of 850 patients from the ASPIRE Registry were diagnosed with CTEPH over the 10-year study period. Seventy-three of these patients originated from the city of Sheffield. The diagnostic rate for residents from Sheffield with CTEPH was 13.2/million/year (based on a referral population of 544,600). Diagnostic rates were significantly lower for non-Sheffield residents at 3.9–5.2/million/year (based on a referral population of 15-20 million), (Figure 18).

Figure 18 ***Central figure illustrating key results including population-based CTEPH diagnostic rates and PEA rates***



Created with BioRender

Abbreviations: PE= pulmonary embolism, RHC= right heart catheterisation, CTEPH= chronic thromboembolic pulmonary hypertension, PH= pulmonary hypertension, mRAP= mean right atrial pressure, mPAP= mean pulmonary artery pressure, CI= cardiac index, PVR= pulmonary vascular resistance

Of 73 Sheffield patients, 41 were referred from the PE clinic and 32 were referred directly with suspected PH. Of Sheffield patients directly referred with suspected PH, 26 (81%) were referred from specialist out-patient clinics, primarily cardiology and respiratory and 6 (19%) were referred as in-patients, usually with decompensated right heart failure.

Patients diagnosed with CTEPH from the PE clinic had a shorter duration of symptoms preceding their diagnosis of CTEPH (median 6 months (IQR 7)) than those referred with suspected PH (median 12 months (IQR 12) $p<0.01$). Patients diagnosed with CTEPH from the PE clinic also had less severe pulmonary haemodynamic disease (PVR 4.6±2.4 WU versus 8.3±4.6 WU, $p<0.01$) and significantly better exercise tolerance (ISWT 305±218m versus 200±189m, $p<0.05$) than those referred with PH from outside Sheffield (Table 10). There was no significant difference in pulmonary haemodynamics between Sheffield and non-Sheffield residents referred with suspected PH ($p>0.05$, Table 10).

Forty-four percent of non-Sheffield residents referred with suspected PH who were diagnosed with CTEPH underwent PEA surgery, whereas 27% of Sheffield residents diagnosed with CTEPH were operated. Nonetheless, population-based PEA rates were significantly higher for Sheffield residents at 3.6/million/year compared to 1-7-2.3 /million/year for non-Sheffield residents. BPA was commissioned as a national service in the UK in 2018 and at the census date no patients from the Sheffield PE clinic had undergone BPA.

Discussion

In the real world setting we have shown that the introduction of a dedicated PE follow-up pathway identifies patients with a shorter duration of symptoms, less exercise limitation and less severe CTEPH than patients presenting with PH, whilst increasing population-based CTEPH diagnostic and PEA rates. Specifically, we have shown that diagnostic rates for CTEPH of 13.2/million/year and PEA rates of 3.6/million/year are achievable. In addition, at the time of acute PE diagnosis the absence of major transient risk factors for thromboembolism and CT features of PH and chronic thromboembolism are risk factors for a subsequent diagnosis of CTEPH.

Incidence and outcome of CTEPH following acute PE

In this study patients received a gold standard diagnosis of CTEPH, undergoing multimodality imaging, RHC and multidisciplinary team discussion in a centre experienced in CTEPH management. Our population was unselected, consecutively enrolled and therefore representative of real-world data with a significantly longer duration of follow-up than most previous studies. The cumulative incidence of CTEPH reported in the literature ranges from 0.1- 9.1%. [292–295, 298] Recently, the FOCUS study reported a 2-year incidence of CTEPH of 2.3% which is similar to the 1.89% reported in this study. [228] We have also observed, for the first time, that patients diagnosed with CTEPH from the index PE event, have a significantly worse survival than patients without CTEPH, highlighting the importance of early assessment for CTEPH following acute PE (Figure 16).

Population-based CTEPH diagnostic and PEA rates

By employing an integrated pathway for acute PE follow-up we observed high population-based diagnostic rates for CTEPH (13.2/million/year) in Sheffield residents, higher than the 3.9-5.2/million/year in non-Sheffield residents and 4-7/million/year reported in an epidemiological analysis from Europe, Japan and USA. [299, 302] The population of patients diagnosed with CTEPH from the PE clinic were older with more comorbidities than patients referred with suspected PH, however they also had a better exercise capacity and less severe pulmonary haemodynamics. Our results suggest if a similar standardised approach to acute PE follow-up was adopted elsewhere then one would expect to see CTEPH diagnostic rates 2-3-fold higher than currently observed. For Sheffield residents we also report high population-based rates for PEA at 3.6/million/year, significantly higher than the 0.9/million/year PEA rate estimated in the USA, 1.7/million/year in Europe [299, 302] and 2.2/million/year noted in the UK national audit of PH during the duration of this study 2010-2020. [300]

Characteristics and management of CTEPH identified in the PE clinic versus patients referred with suspected PH

Although we observed PEA population-based rates for Sheffield residents during the 10-year duration of this study to be significantly higher than previously published international rates, a significantly lower proportion of Sheffield residents with CTEPH from the PE clinic (27%) proceeded to PEA than non-Sheffield residents referred with suspected PH (44%). Patients attending the PE clinic had milder haemodynamic abnormalities and despite being older, a better exercise capacity than patients referred with suspected PH. It is likely these factors impacted on the 39% of patients who

declined surgery. Where strategies are in place to identify patients with CTEPH following PE, our data suggests that significant numbers of patients, particularly those who are older with comorbidities, are more likely not to undergo PEA. We have previously shown that patients with surgical disease who decline surgery have a worse prognosis than patients who undergo pulmonary endarterectomy, [303] highlighting that further work needs to be performed to understand factors influencing treatment decisions and identify the most appropriate regimen tailored to the individual patient. Our data also demonstrates the importance of how we model data to estimate the need for surgical provision. Although we can achieve CTEPH diagnostic rates 2-3-fold higher than currently reported, in the UK where population-based endarterectomy rates are the highest in the world, it is likely that increases in surgical capacity will be more modest.

Sheffield PE follow-up clinic

Our data supports a targeted approach whereby patients are carefully assessed at 3-4 months following acute PE, (Figure 13). Those who are at low risk of CTEPH or symptomatically well are discharged with advice and asked to represent if increasingly symptomatic. We also confirm the findings of a previous study [298] that patients with no major transient risk factors present at the time of the index acute PE have a significantly higher risk of CTEPH. Computed tomography features of CTEPD and CTEPH were frequently seen at diagnosis in patients who developed CTEPH during follow-up, confirming the results of a smaller previous study which identified features of CTEPD in 4 out of 7 patients subsequently diagnosed with CTEPH. [297] This highlights the importance of reviewing the CTPA at the time of the index PE in addition

to identifying risk factors for the development of CTEPH. Whereas previous investigators have focussed on features of CTEPD at the index PE (dilated bronchial arteries, arterial webs or bands, attenuated blood vessels and mosaic parenchymal perfusion pattern) we have shown the value of also looking at CT features of PH. All 3 CT features of PH were present (PA≥30mm, RV:LV ratio≥1 and RVOTH≥6mm) in combination with at least 2 out of 4 features of CTEPD in 19% of patients who developed CTEPH and in 0% of patients who did not develop CTEPH at follow-up. Whereas enlargement of the PA and an increase in RV:LV ratio were seen in 67% and 78% of patients who developed CTEPH compared to 31% and 36% of patients who did not develop CTEPH, respectively, RVOTH and all 3 features of PH were present in 44% and 36% of patients who developed CTEPH and in only 8% and 6% of patients, suggesting that the presence of RVOTH and all 3 features of PH may aid identification of patients at significantly increased risk of developing CTEPH. These CT features are easier to appreciate by the non-specialist radiologist and are also amenable to automated analysis using AI approaches. [304] In contrast, features of CTEPD are more challenging to identify by the non-specialist and to date have eluded automated diagnosis using AI approaches. A standardised approach to acute PE follow-up also allows for the introduction of long-term anticoagulation for those at increased risk of recurrent VTE in addition to providing counselling and education for patients (Figure 13). Long-term anticoagulation would be expected to reduce recurrent VTE and may reduce the subsequent risk of CTEPH, if offered to suitable patients with no major transient risk factors for their acute PE.

Limitations

This study is limited by its single centre and retrospective design and the findings would benefit from external validation. A significant number of patients (41%) diagnosed with acute PE during the conduct of this study were not followed up in the PE clinic; this included patients with malignancy who were followed in a separate pathway and those deemed to be frail by the referring physician. It is possible that some of these patients may develop CTEPH in the future and present with PH. In addition, some patients seen in the PE clinic may have become symptomatic and not sought medical advice and have undiagnosed CTEPH. Nonetheless, our results do establish minimum population-based rates of CTEPH diagnosis and PEA that are likely representative of those that can be achieved in large metropolitan districts. Our PE clinic includes both a thrombosis expert and a respiratory physician and the results may not be directly translatable to alternative types of PE clinic provision.

Conclusion

In conclusion, in the largest study to report on CTEPH diagnostic rates in patients undergoing systematic assessment following an acute PE, we have demonstrated that a single follow-up visit can identify patients earlier with less severe CTEPH and can achieve higher population based CTEPH diagnostic and pulmonary endarterectomy rates that seen in previous studies. In addition, at the time of the index PE event the absence of major transient risk factors for venous thromboembolism and CT features of PH and chronic thromboembolism, should alert the physician to the possibility of CTEPH. This study adds to the growing evidence supporting structured follow-up of patients with venous thrombo-embolic disease.

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Chapter 5: Discussion

In this thesis I have explored the potential methods of improving the diagnosis and assessment of PH by optimising clinical pathways.

Firstly, I have examined non-invasive biomarkers that reflect right ventricular function (NT-proBNP and BNP) and I have shown that the *laboratory test* is repeatable, stable when posted, the impact of exercise testing minimal and *point of care testing* is feasible and able to accurately risk stratify patients with PAH. In doing so I hope this will provide physicians with reassurance that will allow for more rapid on-the-day risk assessment and aid the implementation of more remote approaches to the clinical assessment of patients.

Secondly, I have examined the impact of the introduction of a new clinical pathway to aid early detection of PH as part of a multifaceted approach to the assessment of patients following acute PE. I have demonstrated that patients with CTEPH can be diagnosed earlier, with less severe haemodynamic disease. Such a pathway is practical to implement, and diagnostic rates are similar to clinical studies, however, they can be achieved with a single visit and without the need for multiple repeat investigations and assessments. Interestingly I have also highlighted risk factors for CTEPH including features on CTPA which are more amenable to AI assessment than features of CTEPD.

5.1 Using blood biomarkers reflective of RV function to aid remote risk assessment and aid decision making at the time of the clinical assessment: **what we have learnt and on-going work**

Risk stratification of patients with PAH is recommended in international guidelines to aid treatment and counselling of patients. Integral to all risk stratification approaches are the natriuretic peptides, NT-proBNP and BNP. When used in combination with other clinical parameters including symptoms, WHO FC, imaging metrics and pulmonary haemodynamics, populations of patients can be classified into different risk strata that can predict survival both at diagnosis and follow-up. The aim is to move patients into a lower risk score by increasing treatment if appropriate to ideally obtain a low-risk status or intermediate-low risk status if the patient has comorbidities. However, despite the key role of BNP/NT-proBNP as a biomarker there was previously very limited data on repeatability in patients with PAH and no data on the use of POCT.

Therefore, in chapter 3, we investigated an alternative quicker form of processing by examining the reliability of a near-patient test for NT-proBNP and BNP. The aim was to demonstrate if this could help to improve the assessment of PAH patients at the time of clinic assessment by optimising this clinical pathway. In addition to this we explored a clinical pathway to optimise the remote monitoring of patients with PAH by examining the effect of a pre-processing time delay on reliability of NT-proBNP measurement in laboratory samples.

In terms of assessing the repeatability of sampling I was keen to ensure that we benchmarked the performance of the test, to the ability to accurately risk stratify patients, to assess the performance of the test in clinical practice. There are several risk stratification models that have been proposed to aid assessment including the French Pulmonary Hypertension Network registry risk equation [271], the PH

connection equation [273], the Scottish composite score [275], the REVEAL risk equation [159, 305], and the 2015 and 2022 ESC/ERS PH guidelines risk tables [19]. For this study we chose to use the COMPERA 2.0 risk stratification model, which subdivides patients according to a number of parameters into low, intermediate and high risk at diagnosis and low/intermediate-low/intermediate-high/high risk categories at follow-up. [160] The COMPERA risk stratification score initially was a 3-strata score however, further refinement for the cut-off levels for functional class, 6MWD and BNP/NT-proBNP at follow-up, was found to be more sensitive to prognostically relevant changes. [160]. I elected to utilise to the 4 risk strata model as I felt it would be more challenging than the 3 risk strata model. Using this approach I was able to demonstrate in Chapter 3 that BNP and NT-proBNP testing was repeatable by performing repeat testing after 1 hour of resting, that exercise testing did not impact on the risk stratification assessment for either laboratory or point of care testing and, for laboratory testing, that a delay of processing of samples that included handling by the postal service did not impact significantly on the performance of NT-proBNP.

5.1.1 Limitations

However, there are several important caveats. Firstly, with respect to POCT, a number of NT-proBNP POCT test kits failed the quality control process, due to the impact of temperature. For individuals performing these tests it is essential that they are aware that the test kits need to be stored in a fridge and only allowed to reach room temperature just before use. Secondly, during the study a number of postal samples were not processed. To ensure a robust remote service, oversight would be required to confirm that samples were received and although we have shown stability up to 6 days, to assess the effect of more prolonged delays, further studies are required.

5.1.2 Future work

A potential delayed rise in NT-proBNP and/or BNP following exercise may have been missed in this study as repeated samples were taken immediately after the ISWT. Therefore, by repeating NT-proBNP and BNP at several time intervals post ISWT should allow for capture of any potential rise, and this is further work that is planned. We are also planning to implement a pilot study using the POCT test in our outpatient clinic to assess the impact on treatment decisions on the availability of on the day NT-proBNP result. We also plan to conduct a further trial of postal laboratory NT-proBNP for remote monitoring of patients, initially by enrolling patients who are prescribed ERAs and already return regular blood samples for liver function test monitoring (n=1000), paying particular attention to the number of samples received and processed in the laboratory.

In addition to studying the repeatability of NT-proBNP and BNP, I am planning to explore how the addition of cardiac MRI to NT-proBNP can aid risk stratification in patients with PAH. I have built a large dataset of over 1000 patients who have had NT-proBNP sampling and CMR since 2018. I will also use this database to examine the relationship between NT-proBNP and WHO FC, ISWT, Emphasis 10 (a PH specific quality of life score) and the impact that common clinical factors such as impaired renal function, atrial fibrillation and diabetes have upon the ability of NT-proBNP to aid risk stratification.

5.2 Introduction of a new clinical pathway for patients post PE to aid early diagnosis of CTEPH: **what we have learnt and ongoing work**

Across all forms of PH, the diagnostic criteria and therapeutic options have evolved significantly over the last 20 years. Despite a broader range of therapies in PAH, the 3-year survival is estimated to be between 54.9% and 75% in patients with PAH. [272, 274, 306] Studies suggest that early diagnosis of patients with PAH and early institution of therapies may result in improved long-term outcomes [271, 272, 307, 308]. Despite increased survival for patients with PAH with the advancements of treatments, the time delay from symptoms to diagnosis remains the same, at around 2 years in PAH. [309] For patients with CTEPH, the international CTEPH Registry, demonstrated there was a median delay of 13 months in diagnosis of CTEPH from a patients initial PE. [310] This potentially reflects poor awareness of the disease and insufficient screening and diagnostic tests. [252, 310–312] Importantly, post-hoc analysis of the Registry revealed that patients with CTEPH in the highest third of diagnostic delay had a 47% higher predicted probability of death. [310, 313] Furthermore, identifying patients who are surgical candidates has an important impact on survival. Previous studies have demonstrated a 3-year survival for all CTEPH patients of 84%, however in operated patients this increased to 90%. [314]

Screening and early detection programs are already established in patients with systemic sclerosis who are at risk for the development of PAH, screening this population has allowed for identification of patients with milder forms of the disease, therefore allowing for early initiation of therapies and better survival. [315] The evidence above provides a convincing rational for screening other forms of PH where an ‘at risk’ group can be identified. CTEPH is considered to be a post-PE phenomenon, therefore patients who have had a PE could be described as an “at risk” group for the

development of CTEPH that potentially could be monitored. However, there is no consensus guidance on early detection screening programmes or clinical pathways of how patients should be monitored for the potential development of CTEPH after an acute PE. I therefore examined the influence of a PE clinic on diagnostic rates of CTEPH, with the hypothesis that structured specialist review would lead to earlier and more frequent diagnosis of CTEPH.

In Chapter 4, we highlight that there was a higher proportion of patients with features of CTEPD at the index PE who later went on to be diagnosed with CTEPH than for those patients who did not. However, the more subtle features of CTEPD such as dilated bronchial arteries, arterial webs or bands, attenuated blood vessels and mosaic parenchymal perfusion pattern can be challenging features to identify and to date have also been difficult to identify by AI approaches. [304] Just focusing on features suggestive of PH (PA ≥ 30mm, RV:LV ≥ 1, RVOTH ≥ 6mm) at the time of PE diagnosis maybe a more practical approach when trying to identify patients at higher risk of developing CTEPH. Enlarged PA, increased RV:LV ratio and RVOTH ≥ 6mm were seen in 67%, 78% and 44% of patients who developed CTEPH compared to 31%, 36% and 8% of patients who did not develop CTEPH, respectively. Features of CTEPH are easier to be interpreted by AI analysis [304] therefore providing a potential for further work. An AI tool could be created including specific CTPA features and features from the history to predict potential at-risk patients who could be followed up more closely following their index PE.

We also highlighted that patients with CTEPH had a worse prognosis than those without. This finding is to be expected. [314] A more detailed survival analysis would have ideally been performed taking into account factors such as age and comorbidities, however, the nature of the database for the patients who attended the

PE clinic was very limited. In future work, a prospective study should be designed that systematically assesses patients who are diagnosed with PE, collecting more detailed phenotypic data, including a comparison of radiologist-scored scans with the assistance of AI tools, and provide follow-up outcomes of the PE cohort of patients.

Importantly, we found diagnostic rates for CTEPH that were 3-times higher in the Sheffield population than for the non-Sheffield population, and the rates were higher than previous rates published to date. [299, 302] While diagnostic rates were high, there were also interesting differences in the rates of surgical intervention. Although numbers were small, the percentage of patients having PEA surgery was less in the Sheffield population than for the non-Sheffield patients (27% and 44% respectively). However, population rates were higher for the Sheffield population compared to the non-Sheffield population (3.6 and 1.7-2.3/million/year respectively). As the Sheffield cohort was older with milder disease, other factors such as comorbidity and age might have influenced their decision of whether they would like to be assessed or proceed for PEA surgery. It would be expected that patients with more severe disease and poorer quality of life would undergo PEA, whereas those with milder disease may opt for a more conservative approach. Therefore, although we can treble CTEPH diagnostic rates this is unlikely to translate into a trebling of the need for PEA. If the numbers were projected on to UK PEA rates based on 27% of CTEPH patients having PEA with a CTEPH diagnostic rate of 13.2/million it would translate into 243 PEA's/year in the UK. This would represent in a 62% increase in the UK provision for PEA surgery. Currently in the UK around 150 PEA's are performed per year, and this is the highest published population based surgical rate in the world. [146] Therefore, this is an important point in planning services for the UK population in the future.

Of note also, in the Sheffield CTEPH patients, no patients underwent BPA during the study period. This reflects practice in the UK at the time of this study (2010-2020) when only a small number of patients in the UK underwent BPA. BPA was only nationally commissioned by the NHS in 2018 although it had been practised albeit in relatively small numbers since 2014. In the UK in 2021-22 only 24 BPA procedures were performed in total. However, as BPA becomes increasingly more frequent, it would be interesting to examine the types of patients who are accepted and declined for this procedure and patient outcomes.

5.2.1 Limitations

Although this is the largest study reporting upon the outcomes of a PE clinic and diagnostic rates, it is limited by including data from a single PH centre in a retrospective approach.

During the study period 20,494 patients from Sheffield underwent CTPA and 3289 (16%) of these patients were diagnosed with PE. Of these patients diagnosed with PE, 1956 (59%) were seen in the PE clinic. Therefore, a large proportion of patients (41%) who had an index PE in Sheffield did not attend the Sheffield PE clinic. Some of this deficit is due to patients with PE occurring in the context of active malignancy, as these patients were followed up on a separate pathway and those deemed too frail who were not referred. The PE pathway was instituted as a hospital guideline in 2010. It is likely that limited awareness of the clinic in the earlier years played a role in reduced referral numbers.

The diagnostic rates reported in this study are substantially higher than has been previously reported. There may be some Sheffield PE patients that were either not seen in PE clinic or were seen in PE clinic but were discharged who went on to develop

or have undiagnosed CTEPH. Therefore, the diagnostic rates we have reported despite them being high, do suggest the minimum rates achievable.

As referrers did not consistently state whether patients had been referred from a local PE clinic, it is not possible to calculate the proportion of patients from outside Sheffield who followed a structured review pathway prior to referral to Sheffield pulmonary vascular disease unit. Although an increasing number of PE follow-up clinics have been commissioned in the last 5 years, the proportion of patients referred from a structured PE clinic in our study was likely to be small.

5.2.2 Future work

As touched upon in this discussion, further work should include a prospective study examining patients who are seen in PE clinic, detailed clinical information should be recorded, CTPAs at the time of patient's index PE's should be analysed to provide a more accurate picture of the CTPA features and incorporation of radiological AI techniques. This would then allow for exploring the development of a risk score that could be applied to patients who are being seen in an acute PE follow-up clinic after their index PE. Based on this work, factors that could be considered are CTPA findings suggestive of CTEPH or CTEPD, history including transient risk factors and ongoing symptoms following their PE.

5.3 In conclusion

In this thesis, the optimisation of assessment of patients with PH was explored by examining POCT for NT-proBNP and BNP. It was demonstrated that POCT can provide an alternative to laboratory testing and has the added value of being quick and easy to process and available at the time of the clinical consultation allowing for enhanced clinical assessment of patients with PAH. In addition, a possible remote monitoring pathway was explored by demonstrating the stability of postal laboratory NT-proBNP despite a pre-processing time delay, providing data to support incorporation of postal NT-proBNP in remote clinical assessments of patients with PH.

Furthermore, the importance of improving the diagnosis of patients with PH was demonstrated by studying the outcomes of a follow-up PE clinic. This is the largest study reporting on CTEPH diagnostic rates and demonstrated that a single follow-up visit identified patients earlier with less severe disease. In addition, it was noted that at the index PE event, the absence of major transient risk factors for venous thromboembolism and CT features of PH and chronic thromboembolism, are indicators that patients may go on to develop CTEPH. This work provides robust evidence that the optimal clinical pathway for patients who have a PE is for them to be followed-up in a dedicated PE clinic.

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