



# BNP/NT-proBNP in pulmonary arterial hypertension: time for point-of-care testing?

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**Despite the widespread use of BNP and NT-proBNP in pulmonary arterial hypertension there are limited data on the standardisation of sampling and no data on the use of point-of-care testing and its potential value in clinical management** <https://bit.ly/2Xxm9uV>

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**ABSTRACT** Despite the advent of new therapies and improved outcomes in patients with pulmonary arterial hypertension (PAH), it remains a life-shortening disease and the time to diagnosis remains unchanged. Strategies to improve outcomes are therefore currently focused on earlier diagnosis and a treatment approach aimed at moving patients with PAH into a category of low-risk of 1-year mortality. B-type natriuretic peptide (BNP; or brain natriuretic peptide) and N-terminal prohormone of BNP (NT-proBNP) are released from cardiac myocytes in response to mechanical load and wall stress. Elevated levels of BNP and NT-proBNP are incorporated into several PAH risk stratification tools and screening algorithms to aid diagnosis of systemic sclerosis. We have undertaken a systematic review of the literature with respect to the use of BNP and NT-proBNP in PAH and the use of these biomarkers in the diagnosis and risk stratification of PAH, their relation to pulmonary haemodynamics and the potential for point-of-care testing to improve diagnosis and prognosis.

## Introduction

Pulmonary arterial hypertension (PAH), or World Health Organization (WHO) Group 1 pulmonary hypertension (PH), is a rare, progressive condition characterised by endothelial dysfunction and vascular remodelling, leading to increases in mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR) and ultimately right ventricular (RV) failure. PAH may occur sporadically (idiopathic PAH) but may also be drug induced or associated with other conditions, such as connective tissue disease, congenital heart disease, portal hypertension or HIV infection [1–3]. Current guidelines require patients to have a right heart catheterisation (RHC) measured mPAP  $\geq 25$  mmHg, pulmonary artery wedge pressure  $\leq 15$  mmHg and a PVR of  $>3$  WU at rest, in the absence of other causes [1]. Recently, the 6<sup>th</sup> World Symposium on Pulmonary Hypertension Task Force proposed a new definition of mPAP  $>20$  mmHg and PVR  $\geq 3$  WU [4].

Symptoms and survival are primarily related to the development of RV dysfunction and include breathlessness, fatigue and, in severe disease, fluid retention and syncope [5, 6]. The estimated prevalence of PAH reported by national registries ranges between 10 and 52 cases per million worldwide [7].

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Over the past two decades, there have been significant improvements in patient outcomes [8]. Treatment of PAH primarily encompasses pharmacological and lifestyle interventions [1]. Current pharmacological therapies target three distinct biological pathways (prostanoid, endothelin and nitric oxide), with evidence for superiority of combination therapy over monotherapy [1]. 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.

Current approaches to risk stratification rely on assessments of symptoms, exercise capacity and RV function. There are a number of risk stratification scores, including REVEAL 2.0 [9] and scores developed by European registries based on the European Society of Cardiology/European Respiratory Society (ESC/ERS) recommendations [1, 10].

For assessment of RV function, cardiac magnetic resonance (CMR) imaging is the gold standard [11–13], but cost, availability and until recently the paucity of data on its value in risk stratification [13] have limited its use. Active B-type natriuretic peptide (BNP; or brain natriuretic peptide) and the functionally inert N-terminal prohormone of BNP (NT-proBNP) are well-established clinical biomarkers used in PAH and other cardiovascular disorders, such as acute/chronic heart failure and are used as surrogate markers of cardiac function [1, 14, 15]. NT-proBNP is also used to screen for PAH in patients with systemic sclerosis (SSc) [16]. Typically, BNP or NT-proBNP are measured when patients are assessed by their PAH physician and this information is integrated with the results of other investigations. Typically, clinical laboratory-based assays using venous blood samples are used, and results are not immediately available. In contrast, point-of-care testing (POCT) devices can be used to ensure that results are immediately available and can inform decision making at the time of the consultation. In this review we summarise the current evidence for the use of BNP and NT-proBNP in the early diagnosis of PAH and risk stratification, and explore the potential for POCT in PAH.

### Physiology of natriuretic peptides and the role of BNP and NT-proBNP in cardiovascular disease and PAH

Natriuretic peptides are a family 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 [17]. In contrast, BNP has a longer half-life of ~22 min and is more stable, making it more attractive as a clinical biomarker [17]. In a study examining the stability of atrial natriuretic peptide and BNP, blood samples were shown to be stable *ex vivo* for 2 h and 2 days, respectively [18], though some deterioration of BNP can be observed within 4 h at room temperature [19].

BNP is a product of the early-response gene *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 [20]. 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 1) [14, 21–23]. BNP binds to the natriuretic peptide receptor-A, which is primarily expressed in kidney, adrenal, lung, terminal ileum, aorta and adipose tissue [14]. Receptor activation leads to increases in the intracellular secondary messenger cyclic guanosine monophosphate, resulting in vasodilation, natriuresis, aldosterone inhibition and lipolysis [14]. NT-proBNP has no known function but due to its longer half-life compared with BNP (70 min *versus* 22 min) [14] and relative stability in storage [19], it has potential handling advantages as a biomarker.

### BNP/NT-proBNP and comparison with pulmonary haemodynamics, echocardiographic and CMR metrics

It is well established that BNP and NT-proBNP correlate with a number of pulmonary haemodynamic metrics that are associated with survival. Utilising RHC, CHIN *et al.* [24] showed that BNP correlates with right atrial pressure (RAP;  $r=0.66$ ) in patients with PAH. In SSc-PAH, WILLIAMS *et al.* [25] demonstrated that NT-proBNP correlated with mPAP ( $r=0.62$ ), PVR ( $r=0.81$ ), RAP ( $r=0.53$ ) and cardiac index ( $r=-0.50$ ), and also found NT-proBNP to be an independent predictor of survival. In a study of 30 patients undergoing RHC and CMR at baseline and follow-up, changes in NT-proBNP correlated with changes in RAP ( $r=0.49$ ), cardiac index ( $r=-0.45$ ), 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;  $r=-0.81$ ) [26].

Similar findings have been reported using echocardiographic techniques. In chronic heart failure, TROISI *et al.* [27] found BNP levels to be correlated with TAPSE ( $r=-0.33$ ), RAP ( $r=0.34$ ) and PAP ( $r=0.42$ ). In PAH, GOTO *et al.* [28] reported correlations between BNP levels and transthoracic echocardiography (TTE)-measured systolic PAP when left ventricular (LV) dysfunction was excluded ( $r=0.51$ ).

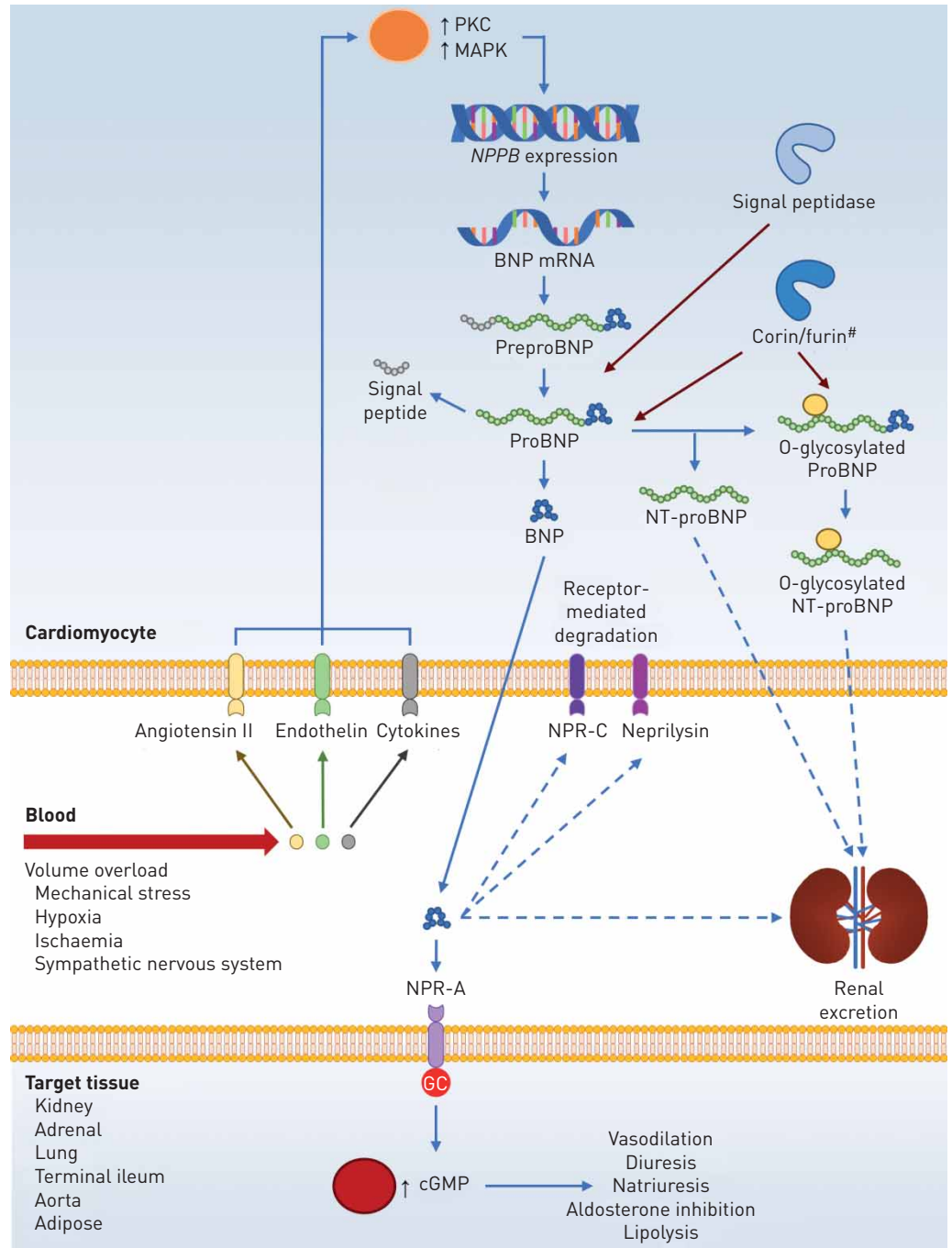


FIGURE 1 Molecular pathways of synthesis and release of B-type natriuretic peptide (BNP) and N-terminal prohormone of BNP (NT-proBNP). aa: amino acid; cGMP: cyclic guanosine monophosphate; GC: guanylyl cyclase; MAPK: mitogen-activated protein kinase; NPR: natriuretic peptide receptor; PKC: protein kinase C. #: candidate enzymes which may produce 29aa or 32aa BNP depending on cleavage site [22, 23].

In a recent study focused on patients with WHO functional class (FC) III PAH who underwent blood testing for BNP, RHC and TTE, BNP levels 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 ( $r=-0.33$ ) and cardiac index ( $r=-0.28$ ) [29]. Using TTE, BNP positively correlated with right atrial ( $r=0.18$ ) and RV enlargement ( $r=0.17$ ), and RV dysfunction ( $r=0.15$ ). In addition, BNP levels correlated with lower 6-min walk distance (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. In PAH, a cohort of 14 patients ( $n=12$  idiopathic PAH) found Spearman's

coefficients of correlation with RVEF for BNP and NT-proBNP of  $-0.54$  and  $-0.61$ , respectively [30]. Similar correlations have been reported by BLYTH *et al.* [31] in PAH and chronic thromboembolic PH ( $n=25$ ), who reported a significant association of NT-proBNP and CMR-RVEF ( $r=-0.66$ ). This 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. In other disorders, a study including 21 patients with minimally symptomatic congenital heart disease-related chronic RV pressure overload found BNP levels to be inversely correlated with RVEF ( $r=-0.65$ ) [32].

### Risk stratification in PAH and the role of BNP/NT-proBNP

Current approaches to risk stratification use a multiparameter approach. The 2015 ESC/ERS guidelines do not explicitly function as a risk calculator, but give reference values for multiple risk parameters that clinicians can interpret in the clinical context [1]. Several studies have validated these ESC/ERS parameters at baseline and follow-up assessment within different European populations [2, 33–36]. These studies have used differing adaptations to the ESC/ERS approach to account for the fact that it is possible for parameters within a single patient to fall into different risk groups.

BOUCLY *et al.* [35] examined a large cohort of patients with idiopathic PAH ( $n=1017$ ) and developed a risk stratification score incorporating WHO/New York Heart Association FC, 6MWD, RAP and CI, quantifying the number of low-risk criteria to predict transplant-free survival in the French Pulmonary Hypertension Registry. Furthermore, they identified that replacing RAP and cardiac index with BNP/NT-proBNP in a subgroup ( $n=603$ ) could also discriminate prognostic groups, an approach which has the potential to reduce the requirement for haemodynamic evaluation. Patients achieving all three low-risk criteria (WHO/ New York Heart Association FC I or II, 6MWD  $>440$  m, and BNP  $<50$  ng-L<sup>-1</sup> or NT-proBNP  $<300$  ng-L<sup>-1</sup>) had 2-, 3- and 5-year survival of 100%, 99% and 97%, respectively. 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 [37].

Further validation for the ESC/ERS approach has been demonstrated in the Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA), which was able to achieve accurate mortality estimates by using an adapted version of the guidelines [2]. By grading each measure to give each patient a single composite risk score, the study demonstrated 1-year mortality rates of 2.8%, 9.9% and 21.2% in low-, intermediate- and high-risk groups, respectively.

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, and has also been validated in multiple studies [9, 38–41]. An update, REVEAL 2.0, has recently been published [9] which, in an independent cohort, 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%, respectively [41]. Additionally, a study using CMR of RV end systolic volume index adjusted for age and sex has been shown to further improve risk stratification for 1-year mortality when used with either the REVEAL 2.0 score or a modified French Pulmonary Hypertension Risk score [13].

An additional factor when considering the clinical practicality of risk stratification is frequency of follow-up. A key study by NICKEL *et al.* [42] in patients with idiopathic PAH demonstrated that follow-up measurements of WHO FC, cardiac index, mixed venous oxygen saturation and NT-proBNP had higher predictive value *versus* baseline for transplantation-free survival, underlying the importance of assessing these variables throughout the course of disease.

### Screening for PAH in systemic sclerosis

In addition to risk stratification, BNP and NT-proBNP can also be used to screen for PAH in connective tissue disease, especially SSc. A study of 109 patients with SSc and NT-proBNP  $>395$  ng-L<sup>-1</sup> showed a positive predictive value of 95.1% (sensitivity 55.9%/specificity 95.1%) and negative predictive value of 56.5% for associated PAH, and changes in NT-proBNP levels were highly predictive of mortality (baseline NT-proBNP: HR=4.82; follow-up NT-proBNP: HR=3.82) [25]. In the DETECT study, which proposed a novel algorithm based on ESC/ERS guidelines and including NT-proBNP in multivariable logistic regression models (estimated coefficient (95% CI) 0.915 (0.308–1.521),  $p=0.003$ ), 19% of SSc patients had RHC-confirmed PAH [16]. The Australian Scleroderma Interest Group (ASIG) has also recently incorporated NT-proBNP into a screening algorithm [43].

### BNP and NT-proBNP considerations for sampling

#### *Physiological characteristics*

RHC is the gold standard for measuring mPAP; however, in clinical practice, the presence of PH is usually first suspected following noninvasive testing. TTE is typically recommended 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 PAP [28]. Imaging investigations such as computed tomography may also suggest the presence of PH by demonstrating pulmonary artery enlargement or RV dilation amongst other features [11].

Elevated BNP/NT-proBNP can indicate several cardiovascular pathologies including LV dysfunction, LV hypertrophy and PAH [1, 14], although other factors that can influence BNP concentrations must be considered and abnormal levels need to be put into clinical context [1, 44]. In addition to being indicators of ventricular stress, BNP and NT-proBNP values are also increased by demographic factors such as higher age, female sex and body weight, as well as the presence of chronic renal failure, type 2 diabetes mellitus, anaemia, pulmonary embolism and acute coronary syndrome [45–47]. In renal failure for example, circulating levels of BNP and NT-proBNP inversely correlate with kidney function, though due to the fact that NT-proBNP is exclusively excreted into urine, the effect is much more pronounced for this biomarker [48].

Standardisation of blood draw is not well established. 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 [49]. Additionally, BRUNS *et al.* [50] noted that time of day may be a factor, 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 [50, 51].

Consensus is also required on the impact of physical exertion prior to sampling and how to account for this. 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·mL<sup>-1</sup> for BNP, which is the recommended acute heart failure threshold [52], and 125 pg·mL<sup>-1</sup> for NT-proBNP), respectively, when measured <24 h after an event [53]. Rapid, transient plasma BNP increases well below 100 ng·L<sup>-1</sup> have also been observed in healthy individuals undertaking short-term maximal exercise, peaking at a mean 30.6±4.7 ng·L<sup>-1</sup> from a resting level of 19.4±2.5 ng·L<sup>-1</sup> immediately after exercise and returning to baseline within 1 h [54].

In cardiovascular disease, small BNP increases in patients after 1 day of 30–50 km walking exercise (median 28.1 pg·mL<sup>-1</sup>, baseline to 35.7 pg·mL<sup>-1</sup>) have been reported, though reported baseline levels were already high relative to individuals with cardiovascular risk factors (3.9 pg·mL<sup>-1</sup>) and healthy controls (5.5 pg·mL<sup>-1</sup>) [55]. Although this is still well below the heart failure threshold, the impact of such increases has not been assessed in patients in PAH where it could conceivably impact on how patients are risk stratified. Similarly, elevated baseline and post-exercise BNP values are reported in individuals with severe LV diastolic dysfunction [56], but not in those with preserved LV ejection fraction [57]. Increases in BNP related to increased physical exertion were also substantially greater compared with individuals with normal diastolic function (96.9 pg·mL<sup>-1</sup> increase *versus* 12.4 pg·mL<sup>-1</sup>) [56].

Decreases in NT-proBNP with endurance exercise, but increases with strength exercise, have been described in sedentary individuals [58]. However, KUTSCH *et al.* [59] in a study of 63 patients, concluded that accounting for exercise-related changes in NT-proBNP did not improve correlation with other surrogate markers of disease.

### Laboratory assays

Clinical laboratory assays have several limitations that can potentially impact the efficacy of patient monitoring [60]. Analysis protocols involve multiple stages, including specific pre-analytical processes that, combined with resource or capacity restrictions in a hospital, mean that time-to-results can take up to several days. This limits the potential use of BNP in the emergency setting, as well as “BNP-guided therapy”, where serial measurements could be used to monitor patients and potentially guide therapy. For example, the need for specialist laboratories prevents monitoring in primary care or at the patient’s home, 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 heart failure [61].

There is no standard protocol for BNP or NT-proBNP sampling and testing. Although risk stratification guidelines recommend specific threshold values to indicate PAH severity (table 1), accuracy and analytical range can vary between 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 [15].

Poor diagnostic concordance between the two biomarkers for ruling in/out heart failure has also been reported, with highly variable levels of the two peptides over time [62]. This can be exacerbated by chronic kidney disease, in part due to elevation of NT-proBNP relative to BNP in these patients. Another study by

TABLE 1 Comparison of European Society of Cardiology (ESC)/European Respiratory Society (ERS) and REVEAL 2.0 prognostic tools

Variable	ESC/ERS guidelines			REVEAL 2.0 risk score calculator <sup>#</sup>		
	Low risk: <5%	Intermediate risk: 5–10%	High risk: >10%	–1 unless indicated	+1	+2 unless indicated
<b>WHO PH Group 1 subgroup Demographics</b>					APAH-CTD	Heritable PoPH (+3) Male, age >60 years
<b>Clinical signs of right heart failure</b>	Absent	Absent	Present			
<b>Comorbidities</b>					eGFR <60 mL·min <sup>-1</sup> /1.73 m <sup>2</sup> or renal insufficiency	
<b>Symptom progression</b>	No	Slow	Rapid			
<b>Vital signs</b>					SBP <110 mmHg HR >96 beats·min <sup>-1</sup>	
<b>Syncope</b>	No	Occasional syncope	Repeated syncope			
<b>All-cause hospitalisations ≤6 months</b>					≥1	
<b>NYHA/WHO FC</b>	I, II	III	IV	I	III	IV
<b>6MWD</b>	>440 m	165–440 m	<165 m	≥440 m (–) 320–<440 m	<165 m	
<b>CPET</b>	Peak $\dot{V}_{O_2}$ >15 mL·min <sup>-1</sup> ·kg <sup>-1</sup> (>65% pred) $\dot{V}_E/\dot{V}_{CO_2}$ slope <36	Peak $\dot{V}_{O_2}$ 11–15 mL·min <sup>-1</sup> ·kg <sup>-1</sup> (35–65% pred) $\dot{V}_E/\dot{V}_{CO_2}$ slope 36–44.9	Peak $\dot{V}_{O_2}$ <11 mL·min <sup>-1</sup> ·kg <sup>-1</sup> (<35% pred) $\dot{V}_E/\dot{V}_{CO_2}$ slope ≥44.9			
<b>BNP/NT-proBNP plasma levels</b>	BNP <50 ng·L <sup>-1</sup> NT-proBNP <300 ng·L <sup>-1</sup>	BNP 50–300 ng·L <sup>-1</sup> NT-proBNP 300–1400 ng·L <sup>-1</sup>	BNP >500 ng·L <sup>-1</sup> NT-proBNP >1400 ng·L <sup>-1</sup>	BNP <50 ng·L <sup>-1</sup> NT-proBNP <300 ng·L <sup>-1</sup>	BNP 200–800 ng·L <sup>-1</sup>	BNP ≥800 ng·L <sup>-1</sup> NT-proBNP ≥1100 ng·L <sup>-1</sup>
<b>Imaging/echocardiography</b>	RA area <18 cm <sup>2</sup> No pericardial effusion	RA area 18–26 cm <sup>2</sup> No or minimal pericardial effusion	RA area >26 cm <sup>2</sup> Pericardial effusion		Pericardial effusion	
<b>Haemodynamics/right heart catheterisation</b>	RAP <8 mmHg CI ≥2.5 L/min/m <sup>2</sup> $S_{VO_2}$ >65%	RAP 8–14 mmHg CI 2.0–2.4 L/min/m <sup>2</sup> $S_{VO_2}$ 60–65%	RAP >14 mmHg CI <2.0 L/min/m <sup>2</sup> $S_{VO_2}$ <60%	PVR <5 Wood units	Mean RAP >20 mmHg within 1 year	
<b>Pulmonary function test</b>					$D_{LCO}$ <40% pred	

ESC/ERS guidelines state that, while not all variables need to be assessed, WHO FC and at least one measurement of exercise capacity (6MWD or CPET) should be taken as a minimum, and assessment of right ventricular (RV) function (BNP/NT-proBNP or echocardiography) is recommended. REVEAL 2.0 includes 11 variables including modifiable and non-modifiable with each score corresponding to a risk for mortality at 1 year. To mirror the ESC/ERS approach the REVEAL score can also be split into three groups (low risk ≤6, intermediate risk 7–8, high risk ≥9) [1, 9]. REVEAL: Registry to Evaluate Early And Long-term PAH Disease Management; WHO: World Health Organization; PH: pulmonary hypertension; NYHA: New York Heart Association; FC: functional class; CPET: cardiopulmonary exercise test; 6MWD: 6-min walk distance; BNP: B-type natriuretic peptide; NT-proBNP: N-terminal pro-hormone of BNP; APAH-CTD: pulmonary arterial hypertension associated with connective tissue disease; PoPH: portopulmonary hypertension; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; HR: heart rate;  $\dot{V}_{O_2}$ : oxygen consumption;  $\dot{V}_E$ : minute ventilation;  $\dot{V}_{CO_2}$ : carbon dioxide production; RA: right atrium; RAP: right atrial pressure; CI: cardiac index;  $S_{VO_2}$ : mixed venous oxygen saturation; PVR: pulmonary vascular resistance;  $D_{LCO}$ : diffusing capacity of the lungs for carbon monoxide. #: add/subtract for each variable to get overall score.

COLLIN-CHAVAGNAC *et al.* [63] compared 10 different clinical laboratory and POCT assays, reporting that median BNP varied between 315 and 526 ng·L<sup>-1</sup> and NT-proBNP between 1020 and 1450 ng·L<sup>-1</sup>. The authors concluded that, while useful diagnostically, none of the tests could be reliably cross compared and

recommended that patients should consistently use the same device model [63]. A UK-based consensus group set up to develop clinical guidance in PAH also recommend users take part in a quality assurance scheme and stick to manufacturer recommendations, given the potential for variation between kits [64].

### Comparison of 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 [1]. A meta-analysis incorporating 48 evaluations of five different assay products in 37 unique cohorts (BNP: 26 cohorts; NT-proBNP: 18 cohorts) found both molecules achieved “excellent” predictive value for excluding acute heart failure at their lower cut-off thresholds [65]. Similar findings have also been reported specifically in POCT assays, with close correlation in regression analysis ( $r=0.93$ ;  $p<0.01$ ) in a single study including 151 patients with structural heart disease [66]. However, there are clinically relevant differences. The advantages and disadvantages of these two molecules for PAH risk stratification are summarised in table 2.

BNP has been shown to correlate better with pulmonary haemodynamics in patients with renal dysfunction as, unlike NT-proBNP, it is captured or cleared by natriuretic peptide receptors or inactivated by neprilysin [1, 48, 67]. Conversely, NT-proBNP is considered more accurate for prognosis and predicting mortality because it integrates renal insufficiency and haemodynamic impairment [1, 67], especially in females and younger patients [68].

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 h, whereas NT-proBNP can reasonably be stored at room temperature for up to 2 days [18, 64, 69]. Use of POCT, however, eliminates this issue. Another potential advantage of NT-proBNP testing over BNP testing is that all commercially available clinical laboratory immunoassays at time of publication, including POCT assays, are based on the same antibodies and calibrators distributed by Roche Diagnostics (Rotkreuz, Switzerland), making assays relatively consistent [20]. By contrast, BNP immunoassays are 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 [20, 70, 71], and these inconsistencies remain when using the same antibodies on different instruments [20, 70, 72]. This variety in sensitivity/specificity is also present in studies specifically evaluating BNP POCT [73].

Cross-reactivity with proBNP-derived peptides can affect BNP and NT-proBNP differently between assays. The entire 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 [74–76], potentially elevating reported values. 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

TABLE 2 Comparison of B-type natriuretic peptide (BNP) *versus* N-terminal prohormone of BNP (NT-proBNP) in clinical practice

BNP	NT-proBNP
Active peptide, inducing compensatory mechanisms for cardiovascular injury/stress	Active function not known, if any
Half-life ~22 min [14]	Half-life ~70 min [14]
Correlates better with pulmonary haemodynamics in PAH [1, 67]	Correlates better with prognosis in PAH [1, 67]
Assays use different antibodies and standard materials (introduces challenges over consistency of results between products and protocols)	Assays based on same antibodies and calibrators (gives relative consistency between products and protocols, but accuracy potentially reduced by glycosylation) <sup>#</sup>
Must not be collected in non-siliconised glass tubes [19]	Glass or plastic tubes can be used [19]
Shorter stability in storage [19]	Longer stability in storage [19]
Assay dependent	7 days at room temperature
Deterioration typically occurs within hours at all temperatures	10 days at 4°C
	≥2 months at –20°C

PAH: pulmonary arterial hypertension. <sup>#</sup>: extent of glycosylation may be influenced by pathology, e.g. increases seen in chronic renal failure, which would underestimate the true NT-proBNP level.

and underestimate the true NT-proBNP concentration [22, 72, 77, 78]. 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 [74, 79]. Pre-treating samples with deglycosylation enzymes has been shown to ameliorate these diagnostic limitations [79, 80], although further validation studies are required before clinical adoption.

While standardisation for BNP and NT-proBNP assays is technically difficult with current technologies, it is feasible that consensus could be reached on a tolerable level of discrepancy [20, 72].

### **Point-of-care testing: what opportunities are there to improve patient outcomes in patients with PAH?**

Between June and December 2019, we carried out PubMed searches for terms and combinations including “pulmonary arterial hypertension”, “BNP”, “NT-proBNP” and “point-of-care testing”, with no restrictions on publication date. While a number of results were found in comparable searches in chronic heart failure [73] and in unexplained dyspnoea, no relevant papers specifically on the subject of POCT in PAH were identified. Despite this, experience from other therapy areas suggests that POCT has the potential to provide a number of benefits including reduced time-to-result and time-to-diagnosis, and ease of handling, together with similar analytical performance, which may lead to improved patient outcomes and clinical cost effectiveness.

#### *Time-to-result*

One of the potential benefits of BNP/NT-proBNP POCT is improved time-to-result, which typically takes 8–20 min depending on the device, whereas clinical laboratory results are typically turned around in excess of 24 h, although at their quickest, dedicated emergency room STAT laboratories can run tests in <1 h [81]. 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. A 2001 study by MAISEL *et al.* [82] describes how in 200 individuals being 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<sup>-1</sup>.

#### *Analytical performance*

The analytical performance of POCT is typically described as either comparable to or slightly lower than clinical laboratory assays, but there is general agreement that POCT is reliable enough to be used as a management tool [60, 83, 84]. In patients with LV dysfunction, for example, there are multiple studies to show POCT assays are comparable to clinical laboratory assays when using the same antibodies and materials [85, 86]. Device-specific studies of BNP/NT-proBNP POCT include: Triage BNP (Quidel; San Diego, CA, USA) [87, 88]; RAMP NT-proBNP (Response Biomedical; Vancouver, Canada) [89]; cobas h232 (Roche Diagnostics) [90–92]; Minicare BNP (Philips; Eindhoven, The Netherlands) [93]; i-STAT (Abbott; Princeton, NJ, USA), which studies noted had good agreement with clinical laboratory despite low precision [88, 94]; PATHFAST NT-proBNP (Mitsubishi Chemical Europe; Düsseldorf, Germany) [95, 96]; AQT90 FLEX (Radiometer; Copenhagen, Denmark) [97]; Alere NT-proBNP (now Quidel) [98] and Alere Heart Check, although lower precision than clinical laboratory was noted [60, 84, 99]; Rapidpia (Sekisui Medical Co.; Tokyo, Japan) [100]; and SHIONOSPOT (Shionogi & Co.; Osaka, Japan) [100] (table 3). POCT assays also typically have a narrower analytical range than clinical laboratory assays, and this may limit their utility in patients with extreme values [66].

#### *Time-to-diagnosis*

In clinical practice, the turnaround time offered by POCT could potentially translate into improved patient outcomes through reducing time-to-diagnosis/prognosis and treatment, especially in the emergency setting when diagnosis is unclear [81]. In one centre that introduced POCT for a number of biomarker assays, time from sample collection to reporting in the emergency department reduced from 70 to 24 min [101]. In BNP POCT specifically, one of the largest trials of its kind (1586 patients), demonstrated that the Biosite (now Quidel) Triage BNP kit was reliable enough for use in the emergency room, proving more accurate than patient history, physical findings or clinical laboratory assays for identifying the cause of dyspnoea [87]. It also performed better than the National Health and Nutrition Examination Survey or Framingham criteria for diagnosis of chronic heart failure [87].

The BASEL study of dyspnoea also confirmed that, while there was conflicting evidence on the cost benefits of BNP POCT in primary care, it did reduce hospital admission rates and hospital stay duration, improved diagnostic certainty and reduced time-to-diagnosis in the emergency room to 63 min *versus* 90 min compared with clinical laboratory assays [86, 102–105]. Another study implementing NT-proBNP and troponin POCT in a single centre reported improved patient outcomes in acute coronary syndrome



TABLE 3 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]
<b>Quidel Triage BNP Test (formerly Alere Heart Check)</b>	BNP	~15 min	2260	0.95 ( <i>versus</i> 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)
<b>Quidel Triage NT-proBNP Test (formerly Alere NT-proBNP)</b>	NT-proBNP	~20 min	100	0.94 ( <i>versus</i> Roche cobas 8000)	KHEZRI [98]
<b>Roche cobas h 232</b>	NT-proBNP	≤12 min	1887	0.97 ( <i>versus</i> Roche cobas e602) [91]	BERTSCH [92] (n=1591) GILS [90] (n=202) HEX [91] (n=94)
<b>Philips Minicare BNP</b>	BNP	≤10 min	347	0.92 ( <i>versus</i> Siemens ADVIA Centaur)	REENEN [93]
<b>Abbott i-STAT</b>	BNP	~9 min	400	0.98 ( <i>versus</i> Siemens ADVIA Centaur) [88]	SHAH [94] (n=150) Ro [88] (n=250)
<b>Mitsubishi PATHFAST</b>	NT-proBNP	<17 min	326	0.99 ( <i>versus</i> Roche Elecsys) [96]	PEETZ [96] (n=90) ZANINOTTO [70] (n=236)
<b>Radiometer AQT90 FLEX</b>	NT-proBNP	11–21 min	77	>0.99 ( <i>versus</i> Roche Elecsys 2010)	LEPOUTRE [97]
<b>Response Biomedical RAMP NT-proBNP</b>	NT-proBNP	~15 min	540	0.98 ( <i>versus</i> Roche Elecsys 2010)	LEE-LEWANDROWSKI [89]
<b>Sekisui Medical Rapidpia</b>	BNP	<15 min	57	0.93 ( <i>versus</i> SHIONOSPOT) <sup>#</sup>	ISHIDA [100]
<b>Shionogi SHIONOSPOT</b>	BNP	~16 min	57	0.93 ( <i>versus</i> Rapidpia) <sup>#</sup>	ISHIDA [100]

BNP: B-type natriuretic peptide; NT-proBNP: N-terminal prohormone of BNP; POCT: point-of-care testing. <sup>#</sup>: Sekisui Medical Rapidpia and Shionogi SHIONOSPOT are both POCT devices compared with each other in this study.

compared with similar hospitals, reducing 30-day readmission rates from 10.4% to 4.2%, and hospital mortality from 15.8% to 9.8%. The authors concluded that “POCT has been critical to the success of the network, but it needs to be implemented within an integrated system of care to produce optimal outcomes” [106].

#### *Ease of handling*

POCT also potentially reduces handling errors by being easier to use and simpler to implement than clinical laboratory assays. Several steps in the clinical laboratory testing pathway can be eliminated, including transport, storage, pre-analytical processing, result validation and return of results to treating clinician, as well as the requirement for multiple patient consultations [107].

Multiple studies have determined that POCT can be used by physicians at all levels, as well as by allied healthcare professionals and by patients/caregivers. One study found that with at least two standardised training sessions (~1.5 h total), GPs were successfully taught to use BNP POCT for heart failure, which improved clinical decision making [108]. 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 [83].

The HABIT study trained patients with heart failure symptoms to measure BNP at home from blood samples. Of note, the ability to obtain daily data also revealed insights into the natural physiology of BNP which fluctuated on a day-to-day basis in specific patients [61]. A further study into patient use of POCT in heart failure achieved measurements that correlated well with those taken by healthcare professionals [99].

#### *Reduced costs*

Although POCT usually has higher initial per-test costs than clinical laboratory assays, net savings can result from reduced time-to-diagnosis/treatment and reduced hospitalisation time [107]. For example, despite slightly lower accuracy, BNP/NT-proBNP POCT was demonstrated to be reliable enough as a prognostic indicator [28], so could potentially lower costs by reducing the need for echocardiography. SIEBERT *et al.* [109] also report that NT-proBNP testing could reduce echocardiography use by up to 58%, reducing overall per patient costs by 9.4%. In this study, shorter time-to-diagnosis also resulted in a modest decrease in serious adverse event rates and patient hospital days. Additionally, a Spanish centre

identified an optimal threshold of  $280 \text{ ng}\cdot\text{L}^{-1}$  using the cobas h232 POCT device to rule out heart failure and subsequently reduced the need for echocardiography by 67% [110].

On a national scale, an NHS audit reported that replacing echocardiography where possible with NT-proBNP testing throughout the UK could save £1.6 million per year [111]. A Norwegian study similarly reported that the 1-year societal costs of heart failure were lowest when NT-proBNP POCT was used (€505) compared with diagnosis by history/clinical findings (€543) and clinical laboratory (€607), with the savings due to fewer GP visits and less use of spirometry [112].

However, 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 dyspnoea triage, it was found that NT-proBNP POCT made no difference to time in hospital, intensive care unit admission rates or mortality [113]. 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 [114].

### What do we need to know before introducing POCT to PAH assessment?

In theory, currently available and well-established POCT devices would be applicable to PAH risk stratification, but clinical evidence of their use in this therapy area is lacking.

In heart failure, diagnosis is typically a binary result that includes/excludes pathology depending on BNP/NT-proBNP thresholds, though borderline values may still require interpretation or further testing. Risk stratification can be more subtle with multiple thresholds (e.g. low, medium or high) and requiring multiple factors to be taken into account. As a consequence, more research is required to determine if the reported lower accuracy of POCT assays may impact on their use in PAH?

The commonly used threshold values in heart failure due to LV dysfunction presentation are  $35 \text{ ng}\cdot\text{L}^{-1}$  for BNP and  $125 \text{ ng}\cdot\text{L}^{-1}$  for NT-proBNP, or  $100 \text{ ng}\cdot\text{L}^{-1}$  and  $300 \text{ ng}\cdot\text{L}^{-1}$ , respectively, in the acute setting [52]. High sensitivity of both clinical and POCT BNP assays at  $\sim 100 \text{ ng}\cdot\text{L}^{-1}$  has been reported [115]. In PAH risk stratification, the ESC/ERS guideline threshold values are higher: BNP: low  $<50 \text{ ng}\cdot\text{L}^{-1}$ , intermediate  $50\text{--}300 \text{ ng}\cdot\text{L}^{-1}$ , high  $>300 \text{ ng}\cdot\text{L}^{-1}$ ; NT-proBNP: low  $<300 \text{ ng}\cdot\text{L}^{-1}$ , intermediate  $300\text{--}1400 \text{ ng}\cdot\text{L}^{-1}$ , high  $>1400 \text{ ng}\cdot\text{L}^{-1}$  [1]. REVEAL 2.0 has similar thresholds (BNP: low  $<50 \text{ ng}\cdot\text{L}^{-1}$ , intermediate  $200\text{--}800 \text{ ng}\cdot\text{L}^{-1}$ , high  $\geq 800 \text{ ng}\cdot\text{L}^{-1}$ ; NT-proBNP: low  $<300 \text{ ng}\cdot\text{L}^{-1}$ , high  $\geq 1100 \text{ ng}\cdot\text{L}^{-1}$ ) [9], though both scores are comfortably within the analytical range of available POCT devices [60, 84, 87–100, 116].

Capillary samples have been shown to offer good correlation with venous samples in several studies ranging from 111 to 187 patients each [60, 93], as well as acceptable reproducibility [117]. 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 testing was performed and correlated with adverse outcomes and was complementary to weight monitoring [61]. The value of such regular monitoring in PAH and whether it could be used to direct treatment and guide diuretic therapy is not known.

### Conclusion

BNP/NT-proBNP POCT has been successfully implemented and is well established in multiple cardiovascular pathologies, including acute and chronic heart failure due to left ventricular dysfunction and dyspnoea. In the majority of studies, POCT has been shown to confer benefits including improved patient outcomes through reduced time-to-diagnosis and cost effectiveness. No studies currently exist in PAH using POCT and further work is required to assess its potential clinical utility.

A key challenge in BNP/NT-proBNP testing, whether clinical laboratory or POCT, is the lack of standardisation between protocols and devices. While POCT provides opportunities to simplify clinical measurement of these biomarkers, more research is needed to standardise approaches to sampling and assess their clinical utility.

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