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ORIGINAL RESEARCH ARTICLE

Right ventricular expression of NT-proBNP adds predictive value to REVEAL score in patients with pulmonary arterial hypertension

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Abstract

Aims The Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) risk scores differentiate survivals in patients with pulmonary arterial hypertension (PAH). However, measurements of N-terminal pro B-type natriuretic peptide (NT-proBNP) in the peripheral blood may not adequately reflect early-stage decompensated heart failure (HF). Given that right heart catheterization (RHC) can facilitate measurements of intracardiac NT-proBNP, in this study our aim was to evaluate the predictive role of right ventricular (RV) NT-proBNP measurements in patients with PAH.

Methods and results We prospectively collected intracardiac blood samples for NT-proBNP measurements from patients diagnosed with World Health Organization Group I PAH during RHC. Clinical information including the aetiology of PAH (idiopathic, connective tissue disease, or congenital heart disease) and REVEAL scores were recorded. The primary endpoint was hospitalization for decompensated HF; median duration of follow-up was 28 months. Among the 62 patients evaluated, 12 reached the designated endpoint. REVEAL risk scores were higher among patients hospitalized for HF. We detected no significant differences in plasma NT-proBNP levels in peripheral circulation, in the right atrium, or in pulmonary arterial blood; however, significantly higher levels of NT-proBNP were detected in the RV in patients diagnosed with PAH. RV NT-proBNP was a sensitive predictor (cut-off value 1500 pg/mL) of subsequent hospitalization for HF. Our findings indicate that RV NT-proBNP levels add predictive value to REVEAL scores with respect to future hospitalization due to HF.

Conclusions Right ventricular NT-proBNP levels combined with REVEAL 2.0 could predict the development of subsequent HF in patients with PAH and may be a potential biomarker.

Keywords NT-proBNP; RV expression; HF hospitalization; Group I PAH

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Introduction

Numerous strategies for risk stratification can be used for the management of patients with pulmonary arterial hypertension (PAH).[1,2] Among them, the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) risk score is applied frequently in clinical practice.[1] Using parameters including the New York Heart Association functional class (NYHA Fc), the 6 min walk test (6MWT), cardiopulmonary capacity, echocardiographic and haemodynamic parameters, as well

as levels of N-terminal pro B-type natriuretic peptide (NT-proBNP) in peripheral circulation, the REVEAL risk score is a well-known and reliable marker for patient follow-up and prognosis of disease.[1] As determined by a *receiver operating characteristic (ROC) curve*, the REVEAL 2.0 score demonstrated a greater area under curve (c-statistic 0.76) than any of the other commonly-used strategies for risk assessment.[1] Also, given the need for timely and regular risk assessment in PAH, Benza *et al*. declared a modified REVEAL Lite 2, which uses only six modifiable and non-invasive variables.[3] Reveal 2.0 is often underutilized due to the need for heart catheterization unlike REVEAL Lite 2 now derived and validated with all non-invasive measures. It could also provide discrimination between patients at low, intermediate, and high risk of 1 year mortality. No matter which versions of risk stratifications, NT-proBNP remains a pivotal biomarker and has been reported to correlate tightly with exercise capacity, haemodynamics, right ventricular (RV) function, and survival in most types of PAH.[4–9] Also, when combined with haemodynamic variations, sequential changes in circulating BNP levels have been associated with the likelihood of positive responses to therapy. Nevertheless, measurements of NT-proBNP, which was mainly secreted from ventricular cardiomyocytes, in the peripheral blood may not reflect decompensated heart failure (HF) at its earliest stages with sufficient sensitivity.[4–6] Given that circulating levels of NT-proBNP are influenced by age, renal function and coexistence of systemic disease, this value may not be an optimal, timely, or reliable indicator of disease prognosis with respect to PAH.[7–9]

Right heart catheterization (RHC) is part of the routine evaluation for PAH[10,11]; this procedure facilitates intracardiac measurements of critical biomarkers, such as NT-proBNP.[6,12] Intracardiac NT-proBNP levels have been used to investigate mechanisms associated with elevated pulmonary vascular resistance (PVR) in patients with left HF secondary to pre-existing pulmonary hypertension.[12] Intracardiac sampling may facilitate a more precise measurement of NT-proBNP synthesis and secretion from cardiomyocytes with limited interference from other tissues.[12] Although the reproducibility and comparability of intracardiac BNP has been previously described, blood samples used for NT-proBNP measurement in those studies have never been taken directly from the right ventricle but from the pulmonary artery (PA) and from the PA-wedge position.[4–6] Given that NT-proBNP is mainly secreted from the cardiomyocytes in ventricles,[4–6] we hypothesized that, among patients undergoing RHC for evaluation PAH evaluation, the intracardiac measurements of NT-proBNP may provide an earlier and more reliable prediction of subsequent decompensation due to HF. Here, our goal was to determine whether the intracardiac evaluation of NT-proBNP could enhance the sensitivity and specificity of the REVEAL risk score assessment over that provided by peripheral circulating levels of this mediator.

Methods

Patients

In this longitudinal prospective study conducted from 2014 to 2019, we enrolled patients with World Health Organization classified Group I (pre-capillary) PAH including idiopathic PAH (IPAH), PAH associated with connective tissue disease (CTD), and PAH secondary to congenital heart disease (CHD). According to European Society of Cardiology/European Respiratory Society guidelines, the diagnosis is defined by findings on RHC, including pulmonary arterial pressure $\geq$ 25 mmHg, pulmonary capillary wedge pressure (PCWP) $\leq$ 15 mmHg, and peripheral vascular resistance (PVR) $>$ 3 Wood units in the absence of other aetiologies such as primary lung disease or chronic thromboembolic pulmonary hypertension.[11] Patients who were hospitalized for right HF were defined as acute RV decompensation and were excluded.

Among the 62 subjects enrolled in this study, 28 were diagnosed with IPAH, 21 were diagnosed with CTD, and 13 were diagnosed with CHD-related PAH. Patients of CHD-related PAH had received surgical or catheter-based occlusion for more than 1 year. Each patient provided informed consent. This study was conducted according to the recommendations of the 1975 Declaration of Helsinki on Biomedical Research involving human subjects and was approved by the local ethics committee (IRB: B-ER-106-056). We collected peripheral, right atrial (RA), RV, and main pulmonary arterial blood samples during RHC for NT-proBNP measurements. RHC and echocardiography were conducted at diagnosis; no PAH-specific therapies including prostacyclin, endothelin receptor antagonist, or phosphodiesterase type 5 inhibitor were prescribed at that time. Tests for functional capacity, including the 6MWT and diffusing capacity of the lung for carbon monoxide, were performed prior to RHC. Clinical information including REVEAL risk scores were recorded. Scores above 8, from 6 to 8, and lower than 6 points were defined as high, intermediate, and low risk, respectively.

Blood sampling and analysis

Blood sampling was performed during diagnostic RHC in treatment-naïve patients with PAH. Ten millilitres of blood was collected in EDTA tubes and then centrifuged at 1300 g for 15 min at 4°C. NT-proBNP levels were measured using a Quidel Triage kit (BIOSITE, San Diego, CA, USA) according to the manufacturer's instructions.

Haemodynamic studies

In addition to heart rate and blood pressure, data collected from the RHC study included RA pressure, systolic, mean

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and diastolic RV pressures, pulmonary arterial pressure, PCWP, and cardiac output, which was calculated by the Fick method. PVR was calculated using the formula of (mPAP-PCWP)/cardiac output.

Echocardiography

Standard echocardiography was performed (iE33, Philips) using a 3.5 MHz multiphase-array probe in accordance with the recommendations of the American Society of Echocardiography.[13] Left ventricular ejection fraction was measured using the biplane Simpson's method. RA area was determined from the apical four-chamber view. PA pressure was calculated according to the combination of RA and RV pressures and the trans-tricuspid flow velocity using the Bernoulli equation. Tricuspid annular plane systolic excursion represents the distance of systolic excursion of the RV annular plane toward the apex. In addition, S′ velocity was measured from the lateral side of tricuspid annulus using tissue Doppler imaging. All the analysed images were acquired in three consecutive cardiac cycles and stored digitally at 50–90 frames per second.

Statistical analysis

Continuous data were presented as the means ± standard deviations or as the medians and interquartile ranges, depending on the nature of the distribution. Dichotomous data were presented as numbers and percentages. Comparisons were evaluated using Student's *t*-tests and non-parametric tests (Wilcoxon signed-rank test) for continuous variables that were and were not normally distributed, respectively. χ^2 tests or Fisher's exact tests were used for the categorical variables as appropriate. *ROC curve analysis was used to compare* RV NT-proBNP and REVEAL with respect to hospitalization for HF. Cox regression analyses were performed to identify the factors that were associated with hospitalization for HF. Due to small sample sizes, Cox regression with Firth's penalized likelihood approach was used to reduce the bias of parameter estimates.[14] The incremental prognostic values of significant factors were assessed on the basis of the log-likelihood χ^2 method. Using the age and gender adjusted hazard ratio (HR), the subgroups analysis was represented as Forest plots. The reliabilities of NT-proBNP measurements were assessed on the basis of 20 randomly selected subjects via the linear regression. The Statistical Package for the Social Sciences (SPSS) software (Version 22.0, IBM SPSS Inc., Chicago, IL, USA) and R (i384, 3.6.1) was used for the statistical analyses.

Results

Demographic characteristics of the enrolled pulmonary arterial hypertension patients

Among the 62 patients enrolled in our study, 12 reached the designated endpoint (*Table 1*). The median follow-up duration was 28 months while the time to event duration was 10 months (interquartile range: 6 to 12 months). There were no significant differences in age, gender, comorbidities including systemic hypertension and diabetes between those that did or did not require hospitalization, although we did identify significantly lower body weights and estimated glomerular filtration rates among patients who developed HF and ultimately required hospitalization. Both groups included patients diagnosed with IPAH, CTD, and CHD; among 28 patients diagnosed with IPAH, only 4 progressed to HF, while among those with CTD, there was a greater tendency (5 out of 21) toward requiring hospitalization for HF. A 48.3% of the patients remained at NYHA Fc II during the entire period of enrolment; we detected no differences with respect to the 6MWT or diffusing capacity of the lung for carbon monoxide between the two groups. With respect to echocardiographic and RHC parameters, there were no significant differences between groups except for lower blood pressure in the cohort of patients that required hospitalization for HF. The median REVEAL scores for patients who did and did not require hospitalization for HF were 2 and 8, respectively. Among patients who required hospitalization for HF, 50% were those identified as high risk (≥9). Likewise, in REVEAL Lite 2, the median REVEAL scores for patients with and without hospitalization for HF were 2 and 6, respectively. Fifty per cent of patients who developed HF were REVEAL Lite 2.0 high risk at baseline (Supporting Information, *Table S1*).

Central measurements of N-terminal pro B-type natriuretic peptide

Notably, levels of NT-proBNP detected in peripheral circulation, in the right atrium, and in the PA were not significantly different from one another, but in contrast, we detected significant higher levels of NT-proBNP in blood sampled from the RVs of patients who required hospitalization for HF compared with those who did not [1726 (1515–4052) pg/mL vs. 214(80–426) pg/mL, $P < 0.01$], respectively (*Figure 1*). To investigate the power of REVEAL scores together with RV NT-proBNP levels with respect to differentiating the risk of HF in this patient cohort, we generated an ROC curve for comparison and determined the area under curve to be 0.56 for peripheral NT-proBNP, 0.91 for RV NT-proBNP, and 0.82 for the REVEAL risk score (*Figure 2*). The determined area under curve of RV NT-proBNP was the maximum value

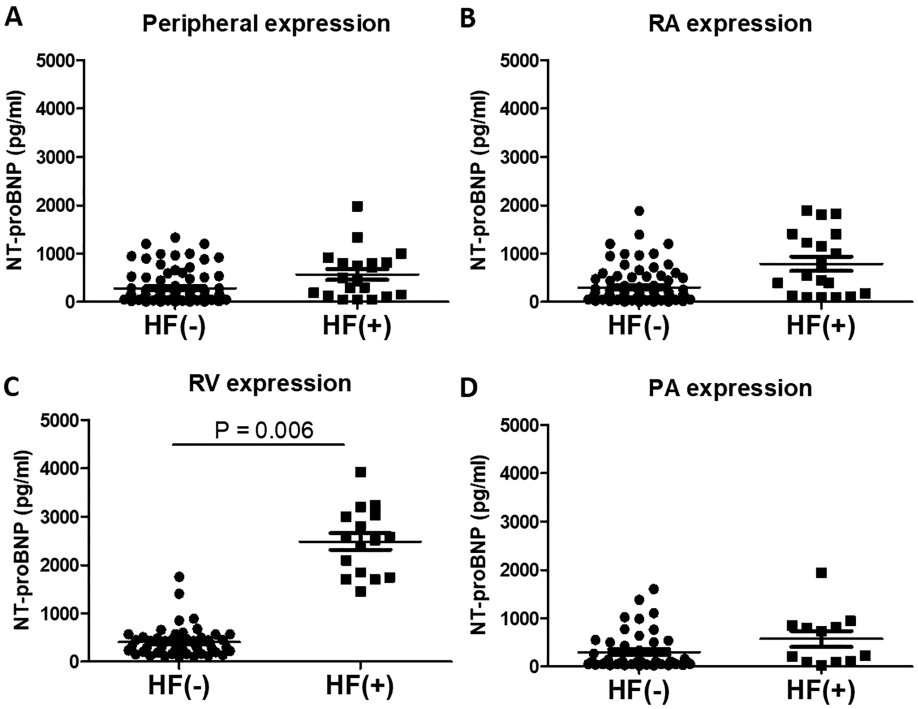
Table 1 The baseline clinical, echocardiographic, functional, and haemodynamic parameters of patients with World Health Organization Group I pulmonary arterial hypertension (PAH)

Parameter	HF hospitalization (−) $N = 50$	HF hospitalization (+) $N = 12$	P value
Clinical parameters			
Age (y/o)	50 (41–61)	51 (40.5–59)	0.94
Gender, *N* (%)	11 (22)	3 (25)	0.70
Body height (cm)	151 (147–162)	152 (147.5–164.5)	0.82
Body weight (kg)	60 (51–72)	50.5 (38.5–58)	0.01
Systemic hypertension, *N* (%)	6 (12)	2 (16.6)	0.65
Diabetes, *N* (%)	5 (10)	1 (8.3)	1.00
Aetiologies			
IPAH, *N* (%)	24 (48)	4 (33.3)	0.52
CHD, *N* (%)	10 (20)	3 (25)	0.70
CTD, *N* (%)	16 (32)	5 (41.6)	0.74
Functional capacity			
NYHA Fc I, *N* (%)	12 (24)	2 (16.6)	0.72
NYHA Fc II, *N* (%)	25 (50)	5 (41.6)	0.75
NYHA Fc III, *N* (%)	11 (22)	3 (25)	1.00
NYHA Fc IV, *N* (%)	2 (4)	2 (16.6)	0.17
6MWT (m)	409.5 (385.0–450.0)	372.5 (340.5–448.5)	0.17
%Predicted DLCO	57.5 (48.0–69.0)	50.5 (38.0–62.0)	0.15
Serologic markers (peripheral blood)			
Haemoglobin (mg/dL)	11.9 (10.9–13.0)	11.5 (10.6–13.5)	0.89
Creatinine (mg/dL)	0.9 (0.8–1.0)	0.9 (0.7–1.3)	0.38
eGFR (mL/min/1.73 m^2)	78.9 (67.9–113.8)	62.0 (54.9–69.0)	<0.01
ALT (IU/L)	24 (15–30)	20.5 (19–26)	0.58
Bilirubin (mg/dL)	0.8 (0.6–1.1)	0.8 (0.7–0.9)	0.68
Na (mmol/L)	135 (130–139)	132 (123–137.5)	0.21
NT-proBNP (pg/mL)	320 (184–480)	316 (192–902.5)	0.50
Echocardiographic parameters			
LVEF (%)	70.0 (68.0–71.0)	71.5 (67.5–76.5)	0.24
RA area (cm^2)	13.95 (12.0–18.0)	15.0 (14.3–15.9)	0.38
TAPSE (cm)	1.7 (1.5–2.1)	2.0 (1.7–2.2)	0.36
S′ (cm/s)	11.0 (10.0–12.5)	11.5 (8.5–13.4)	0.73
PAP (mmHg)	50 (40–72)	61.9 (54–68)	0.17
Pericardial effusion, *N* (%)	18 (36)	5 (41.6)	0.75
Right heart catheterization			
Heart rate (bpm)	81.5 (78–92)	89 (74–96)	0.47
Systolic blood pressure (mmHg)	118.5 (108–128)	117 (105.5–120.5)	0.25
Diastolic blood pressure (mmHg)	71 (66–78)	71 (66.5–75.5)	0.92
RA pressure (mmHg)	10 (7–13)	9 (6.5–10.5)	0.20
mRV pressure (mmHg)	29 (25–33)	34.335 (30–37)	0.04
mPA pressure (mmHg)	33 (29–55)	38 (30.5–57)	0.52
Wedge (mmHg)	11 (10–14)	12 (11–13.1)	0.51
Cardiac index (L/m^2)	3.5 (3–4.2)	3.1 (2.7–4.1)	0.45
PVR (woods)	6.5 (5.6–8)	7.8 (7.075–9.95)	0.07
RA NT-proBNP (pg/mL)	294.5 (164–429)	362.5 (102–901)	0.64
RV NT-proBNP (pg/mL)	214 (80–426)	1726(1515–4052)	<0.01
PA NT-proBNP (pg/mL)	104 (55–335)	105.5(39.5–516.5)	0.97
Medications			
Diuretics, *N* (%)	36 (72)	10 (83)	0.71
Digoxin, *N* (%)	7 (14)	4 (33.3)	0.20
CCB, *N* (%)	3 (6)	1 (8.3)	1.00
Anti-coagulants, *N* (%)	6 (12)	2 (16.6)	0.65
REVEAL risk score			
Median	2	8	<0.01
Range	1 to 9	4 to 12	0.01
High risk (≥9)	10 (20)	6 (50)	0.06
Intermediate risk (7 to 8)	26 (52)	4 (33.3)	0.34
Low risk (≤6)	14 (28)	2 (16.6)	0.71

6MWT, 6 min walk test; ALT, alanine aminotransferase; CCB, calcium channel blocker; CHD, congenital heart disease; CTD, connective tissue disease; DLCO, diffusing capacity for carbon monoxide; eGFR, estimated glomerular filtration rate; IPAH, idiopathic PAH; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; PA, pulmonary arterial; PVR, pulmonary vascular resistance; RA, right atrial; RV, right ventricular; S′, peak systolic tricuspid annular velocity derived by Tissue Doppler imaging; TAPSE, tricuspid annular plane systolic excursion.

Data are expressed as mean ± SD or as number (percentage) for normal distribution. Non-normally distributed variables are presented as median and interquartile range (IQR).

Figure 1 (A) Peripheral, (B) right atrial, (C) right ventricular, and (D) pulmonary arterial expressions of NT-proBNP in Group I PAH patients with (N = 12) or without (N = 50) hospitalization for heart failure. HF, heart failure; NT-proBNP, N-terminal pro B-type natriuretic peptide; PAH, pulmonary arterial hypertension; RV, right ventricular.



of all but without statistical significant difference between REVEAL risk score, P = 0.349.

Cox regression to identify the predictors of hospitalization for heart failure

Using Cox regression analyses, the REVEAL scores and RV NT-proBNP levels were significantly associated with hospitalization for HF as the designated endpoint [HR = 1.54, confidence interval (CI), 1.22–1.94, $P < 0.001$ vs. HR = 1.06, CI, 1.03–1.09, $P < 0.001$, respectively; *Table 2*]. In contrast, age, gender, systolic blood pressure, estimated glomerular filtration rates, haemodynamic variables, and 6MWT failed to differentiate the subsequent hospitalization for HF. Also, without the functional and RHC derived parameters, REVEAL Lite 2 failed to discriminate the subsequent development of HF in patients with PAH in this cohort (HR: 1.39; CI:0.94–2.06, P = 0.103, listed in *Table 2*). Given that REVEAL scores and RV NT-proBNP levels were found to be independently associated with hospitalization for HF, we attempted to determine whether combining REVEAL scores and RV NT-proBNP levels would provide superior prognostic predictions. A −2 log-likelihood test revealed that adding RV NT-proBNP levels, notably those >1500 pg/mL to clinical REVEAL scores provided additional predictive value with respect to hospitalization for HF (*Figure 3*).

The predictive impact of right ventricular N-terminal pro B-type natriuretic peptide in special populations

To determine whether RV NT-proBNP levels are associated with equal predictive values in patients with specific characteristics, we performed a subgroup analysis. Interestingly, although elevated RV NT-proBNP levels were associated with the likelihood of hospitalization for HF among patients with Group I PAH, its predictive value was mainly observed in patients diagnosed with IPAH or CTD. We also determined that predictions based on elevated levels of RV NT-proBNP were most significant in the younger female patients. We also found that the HR increased dramatically in patients with lower NYHA Fc status at baseline and, interestingly, among those with a higher REVEAL risk scores (*Figure 4*).

Reproducibility of N-terminal pro B-type natriuretic peptide measures

To determine the reproducibility of NT-proBNP measurements in the peripheral, RA, RV, and PA, blood sample from

Figure 2 Receiver operating characteristic curve showed area under curve to be 0.56 for peripheral NT-proBNP, 0.91 for RV NT-proBNP, and 0.82 for the REVEAL risk score to differentiate hospitalization for heat failure in patients with Group I PAH. NT-proBNP, N-terminal pro B-type natriuretic peptide; RV, right ventricular.

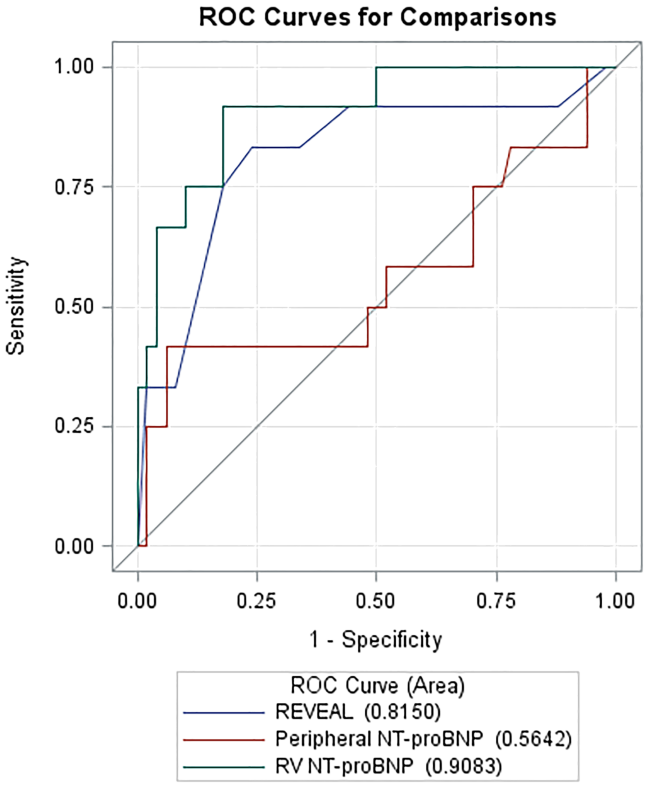


Table 2 Univariate predictors of hospitalization for heart failure

Parameter	Univariate	
	HR	*P*
Age	1.001 (0.956–1.048)	0.968
Gender	0.714 (0.199–2.557)	0.605
Body weight	0.929 (0.877–0.983)	0.011
Systolic blood pressure	1.008 (0.968–1.049)	0.702
eGFR	0.977 (0.954–1.000)	0.050
6MWT	0.996 (0.989–1.004)	0.358
RA pressure (RHC)	0.897 (0.751–1.071)	0.228
RV pressure (RHC)	1.045 (0.990–1.104)	0.111
mPA pressure (RHC)	1.012 (0.982–1.043)	0.451
Cardiac index (RHC)	0.966 (0.627–1.489)	0.876
PVR (RHC)	1.148 (0.942–1.400)	0.171
REVEAL risk score	1.538 (1.220–1.940)	<0.001
REVEAL Lite 2 risk score	1.390 (0.936–2.063)	0.1025
REVEAL risk score > 8	8.427 (2.346–30.274)	0.001
Peripheral NT-proBNP	1.001 (1.000–1.003)	0.082
RV NT-proBNP (per 100)	1.060 (1.034–1.086)	<0.001
RV NT-proBNP > 1500	7.924 (2.206–28.468)	0.002

6MWT, 6 min walk test; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; PVR, pulmonary vascular resistance; RA, right atrial; RHC, right heart catheterization; RV, right ventricular.
Compared with REVEAL risk scores, REVEAL Lite 2 risk scores applies only six modifiable and non-invasive variables including renal insufficiency, NYHA or WHO functional class, systolic blood pressure, heart rate, 6 min walk disease, and BNP/NT-proBNP.[3] Firth's penalized likelihood approach was used to reduce the bias of parameter estimates because of small sample sizes.

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Figure 3 Incremental prognostic value of right ventricular NT-proBNP dichotomized by 1500 pg/mL in predicting hospitalization for heart failure in patients with Group I PAH. NT-proBNP, N-terminal pro B-type natriuretic peptide; ROC, receiver operating characteristic curve; RV, right ventricular.

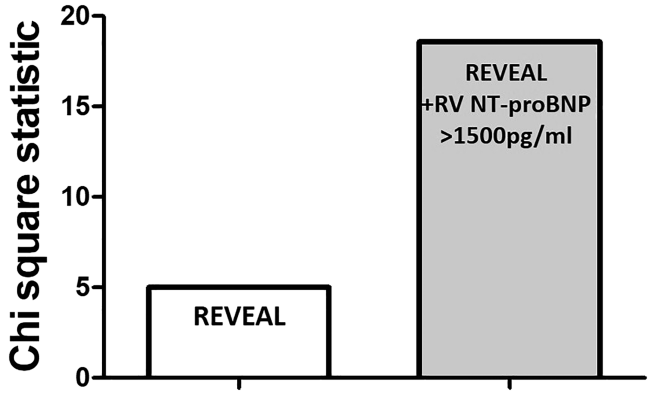
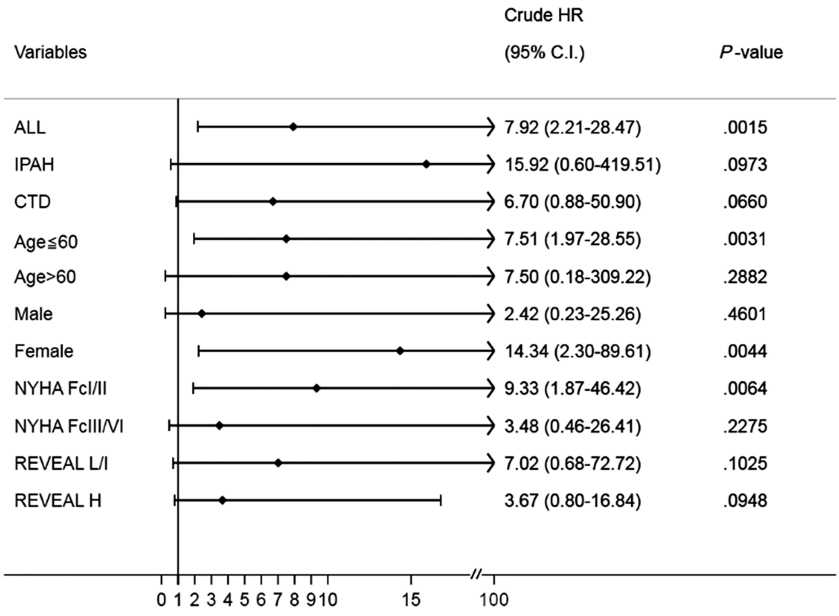


Figure 4 The subgroup analysis of the risks of right ventricular N-terminal pro B-type natriuretic peptide ≥ 1500 pg/mL on hospitalization for heart failure in patients with Group I PAH. CI, confidence interval; CTD, connective tissue disease; HR, hazard ratio; IPAH, idiopathic pulmonary arterial hypertension; NYHA Fc, New York Heart Association functional class.



20 randomly selected subjects were measured twice, and the results showed high reproducibility and reliability (0.9 to 0.969) (*Figure S1*), suggesting robust measure that was highly reproducible.

Discussion

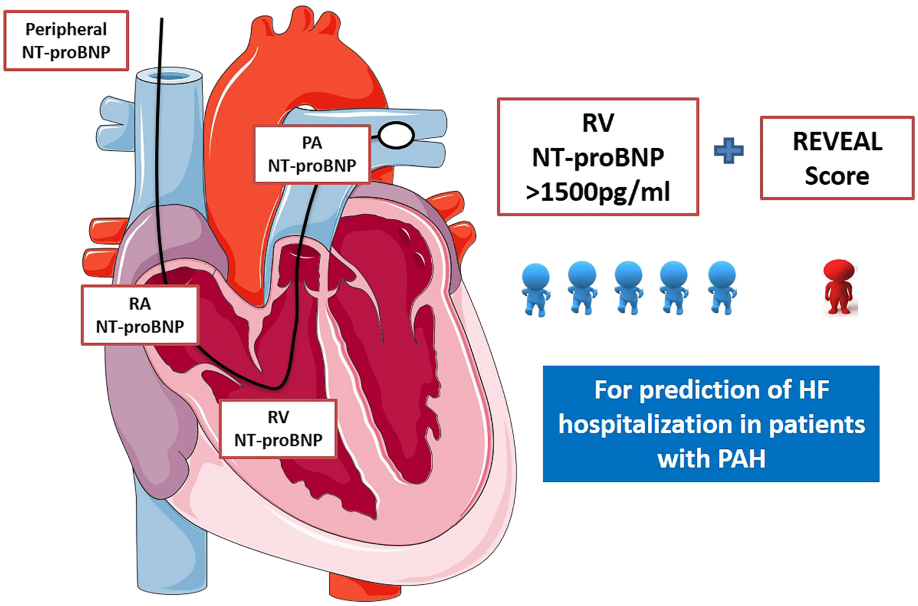
Several potential biomarkers have been considered with respect to the potential to contribute to risk stratification among patients diagnosed with PAH; these include pro-atrial natriuretic peptide, interleukin-6, CT-proET-1, and growth differentiation factor 15.[9,15–18] However, to date, only BNP and NT-proBNP have been included in clinical guidelines.[11] NT-proBNP, a cleavage product of BNP, is secreted from membrane granules of ventricular cardiomyocytes and is an established biomarker for the diagnosis of HF. NT-proBNP levels have also been associated with clinical outcomes in patients with pre-capillary and post-capillary pulmonary hypertension.[19,20] In response to a pressure or volume overload, NT-proBNP levels are an immediate reflection of RV

dilatation and deterioration of systolic function.[21] Parallel to the changes in pulmonary haemodynamics, NT-proBNP levels also correlate with functional parameters, including 6MWT, and are indicators of treatment efficacy.[20,22]

Right heart catheterization remains the gold standard for official diagnosis of PAH, as this methodology permits accurate measurements of PA pressures, PVR, and PCWPs.[10,11] Simultaneous collection of BNP at the time of RHC confirmed the correlation of these values with right heart haemodynamics in patients diagnosed with Group 1 PAH.[23] Despite the ready access to intracardiac and transpulmonary blood samples during RHC, there are no studies that have directly compared intracardiac vs. peripheral levels of NT-proBNP. In patients with high PVR due to left HF, Melenovsky *et al*. reported preserved uptake of transpulmonary BNP, but diminished cyclic guanosine monophosphate release in association with stiffening of both the pulmonary and systemic arteries.[12] Likewise, another study documented the reproducibility of intracardiac and transpulmonary BNP and cyclic guanosine monophosphate measurements in patients with varying types of pulmonary hypertension.[6] Nevertheless, blood samples used in those studies were from the PA and from the PA-wedge position for measurements of NT-proBNP gradients.[6] Given that NT-proBNP were secreted mainly from cardiomyocytes in ventricles, the measurement of NT-proBNP at the RV chamber is speculated to be the most representative marker for timely reflecting the changes of the heart in response to stresses.[1–3] Also, the high pulmonary arterial pressures and con-existing pulmonary regurgitation may attenuate the levels of NT-proBNP measured in the PA. Most importantly, there is only limited evidence regarding the applicability of central NT-proBNP measurements and their association with clinical prognosis. In this study, we identified significantly higher levels of RV NT-proBNP in patients with Group I PAH who subsequently developed decompensated HF. Using Cox regression analysis, the REVEAL scores and levels of RV NT-proBNP were significantly associated with the likelihood of hospitalization for HF as the designated endpoint; measurements of RV NT-proBNP in association with REVEAL scores provided additional predictive power. Although levels of NT-proBNP in peripheral circulation are currently included in risk assessments, our findings indicate the superiority of NT-proBNP measured in the RV chamber for risk stratification of patients diagnosed with PAH (*Figure 5*).

Registry to Evaluate Early and Long-Term PAH Disease Management is a multicentre and observational registry that was originally designed to characterize and to predict risk in a contemporary PAH patient population.[1,24] Using up to 12 clinically relevant variables, REVEAL scores predict survival in diverse PAH populations and provide useful serial survival assessments. Recently, Benza *et al*. reported that the revised REVEAL 2.0 demonstrates better risk discrimination than can be obtained using any of the other risk assessment tools, including the Swedish Pulmonary Arterial Hypertension Register and the Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension.[1] Although REVEAL score is mainly designed for the prediction of mortality, Burger *et al*. also reported that PAH-related hospitalization was associated with relatively more re-hospitalizations

Figure 5 The summary of right ventricular N-terminal pro B-type natriuretic peptide in combination of REVEAL score in predicting hospitalization for heart failure in patients with World Health Organization Group I pulmonary arterial hypertension. HF, heart failure; NT-proBNP, N-terminal pro B-type natriuretic peptide; PA, pulmonary artery; PAH, pulmonary arterial hypertension; RA, right atrial; RV, right ventricular.



at 3 years in the REVEAL registry.[25] REVEAL Lite 2 is only validated to predict survival at 1 year and has not been validated for risk of hospitalization.[3] Nevertheless, in this study, without the functional and RHC-derived parameters, REVEAL Lite 2 failed to discriminate hospitalization for HF for a longer follow-up duration.

According to 2015 European Society of Cardiology/European Respiratory Society guidelines, the cut-off values for NT-proBNP levels for low and high risk are $<$300 ng/L and $>$1100 ng/L, respectively.[11] Different cut-off values have been evaluated for various types of pulmonary hypertension; however, there is still insufficient information with respect to optimal values for intracardiac and transpulmonary measurements of NT-proBNP. In this study, we used the median value of RV NT-proBNP (1500 pg/mL) as the cut-off value; this value specifically differentiated patients and was a strong predictor for the likelihood of decompensated HF. Furthermore, when considered in combination with REVEAL scores, RV NT-proBNP levels $>$1500 pg/mL provided additional predictive values for identifying patients at risk of hospitalization for HF**.**

In subgroup analyses, values of NT-proBNP are influenced by age, gender, and renal function[7,8]; as such, subgroup analyses were performed to evaluate its significance of risk stratification in specific populations. According to our findings, the discriminating power of RV NT-proBNP levels was most significant in younger patients as well as those who are female. Also, echoing previous reports, we observed increasing risk among patients with higher levels of REVEAL scores and higher levels of RV NT-proBNP. Given the limited number of patients who presented with high grade of NYHA Fc (III/VI), we were unable to generate strong predictions regarding RV NT-proBNP levels among patients in this specific subgroup.

Limitations

There are several limitations with respect to this study. First, only 12 of the 62 enrolled patients developed decompensated HF; this factor contributed to a decrease in the statistical power associated with these findings. Second, given that the intracardiac blood flow is not steady but dynamic; the NT-proBNP was speculated to be secreted from the chamber that we measured. Although these intracardiac NT-proBNP levels are possibly mixed with the peripheral blood, the high expression of NT-proBNP at RV implies an increasing RV production or an increased peripheral clearance. Echoing our findings, intracardiac and transpulmonary expressions of biomarkers have been reported to significantly correlate to the haemodynamics in patients with pulmonary hypertension.[12,26] Also, as there are no reference values for RV NT-proBNP, the cut-off value identified here may not be applicable to all patients diagnosed with PAH. This may explain why our results differ from those reported in a previous study which revealed an association between elevated peripheral NT-proBNP levels and an increased risk of mortality. By contrast, in our relatively small patient cohort, peripheral measurements of NT-proBNP failed to differentiate among patients who did or did not develop decompensated HF, but NT-proBNP measurements in the RV chamber strengthened the sensitivity provided by the REVEAL scores. Third, the related peptide, BNP, which functions in natriuresis and vasodilation, has been regarded as a potential therapeutic target. Conversely, despite the fact that it can be measured in circulation, NT-proBNP may only serve as a biomarker. We do not yet know whether central measurements of BNP, as opposed to NT-proBNP, will be superior with respect to risk assessment. Forth, because all the enrolled patients were treatment-naïve, it remains uncertain whether measurements of RV NT-proBNP can contribute to risk stratification during follow-up and/or in response to PAH-specific therapies. Fifth, although we found that RV NT-proBNP is superior to the peripheral NT-proBNP in predicting the subsequent HF hospitalization, whether RV NT-proBNP could replace REVEAL 2 scores requires more validation. Given that monitoring clinical and haemodynamic parameters remains crucial in patients with PAH, we suggested to improve the sensitivity and specificity of REVEAL 2 score by adding RV NT-proBNP. Finally, given the health policy and insurance in Taiwan, the criteria for hospitalization here might not be as strict as that in the other countries. The rate of hospitalization may be slightly higher in Taiwan compared with the others.[27]

Conclusions

Collectively, we report that the NT-proBNP measurement in the RV chamber is additional to that measured in peripheral circulation with respect to predictions associated with hospitalization for HF; hence, RV NT-proBNP may be a superior biomarker for risk assessment of patients diagnosed with Group I PAH. The RV NT-proBNP measurement also augments the power of REVEAL scores with respect to differentiating the risk for developing decompensated HF. As such, intracardiac blood sampling should be considered as an integral part of the RHC procedure used for the diagnosis of PAH.

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Author Contributions

WC and CH conceived the idea for the study. WC, CH, JS, CH, YL, ZC, and JR collected data for the study. WC, YC, and CH analysed the data. WC wrote the first draft. WC, CH, JS, CH, YL, ZC, YC, CH, and JR provided revisions to the first draft. All authors approved the final draft.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Ethics approval

This study was approved by the ethics committee in CMMC (IRB: B-ER-106-056). Written informed consent was obtained from participants.

Data availability statement

Data are available upon reasonable request to the corresponding author.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. The reliabilities of the NT-proBNP measurements. The linear regression between the first and second measurements of NT-proBNP (A) in the peripheral blood (NTBNPperi); (B) in the right atrium (NTBNPRA); (C) in the right ventricle and (D) in the pulmonary artery (NTBNPPA).

Table S1. The REVEAL Lite 2 risk scores of patients with WHO Group I pulmonary arterial hypertension (PAH).

References

1. Benza RL, Gomberg-Maitland M, Elliott CG, Farber HW, Foreman AJ, Frost AE, McGoon MD, Pasta DJ, Selej M, Burger CD, Frantz RP. Predicting survival in patients with pulmonary arterial hypertension: the REVEAL risk score calculator 2.0 and comparison with ESC/ERS-based risk assessment strategies. *Chest* 2019; **156**: 323–337.
2. Raina A, Humbert M. Risk assessment in pulmonary arterial hypertension. *Eur Respir Rev* 2016; **25**: 390–398.
3. Benza RL, Kanwar MK, Raina A, Scott JV, Zhao CL, Selej M, Elliott CG, Farber HW. Development and validation of an abridged version of the REVEAL 2.0 risk score calculator, REVEAL Lite 2, for use in patients with pulmonary arterial hypertension. *Chest* 2020.
4. Casserly B, Klinger JR. Brain natriuretic peptide in pulmonary arterial hypertension: biomarker and potential therapeutic agent. *Drug Des Devel Ther* 2009; **3**: 269–287.
5. Soon E, Doughty NJ, Treacy CM, Ross RM, Toshner M, Upton PD, Sheares K, Morrell NW, Pepke-Zaba J. Log-transformation improves the prognostic value of serial NT-proBNP levels in apparently stable pulmonary arterial hypertension. *Pulm Circ* 2011; **1**: 244–249.
6. Monahan K, Scott TA, Su YR, Lenneman CG, Zhao DX, Robbins IM, Hemnes AR. Reproducibility of intracardiac and transpulmonary biomarkers in the evaluation of pulmonary hypertension. *Pulm Circ.* 2013; **3**: 345–349.
7. Hogenhuis J, Voors AA, Jaarsma T, Hillege HL, Boomsma F, van Veldhuisen DJ. Influence of age on natriuretic peptides in patients with chronic heart failure: a comparison between ANP/NT-ANP and BNP/NT-proBNP. *Eur J Heart Fail* 2005; **7**: 81–86.
8. Srisawasdi P, Vanavanan S, Charoenpanichkit C, Kroll MH. The effect of renal dysfunction on BNP, NT-proBNP, and their ratio. *Am J Clin Pathol* 2010; **133**: 14–23.
9. Kanwar MK. Biomarkers in pulmonary arterial hypertension: moving closer toward precision medicine? *J Heart Lung Transplant* 2020; **39**: 287–288.
10. Duarte AG, Lin YL, Sharma G. Incidence of right heart catheterization in patients initiated on pulmonary arterial hypertension therapies: a population-based study. *J Heart Lung Transplant* 2017; **36**: 220–226.
11. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: the Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Respir J* 2015; **46**: 903–975.
12. Melenovsky V, Al-Hiti H, Kazdova L, Jabor A, Syrovatka P, Malek I, Kettner J, Kautzner J. Transpulmonary B-type natriuretic peptide uptake and cyclic guanosine monophosphate release in heart failure and pulmonary hypertension: the effects of sildenafil. *J Am Coll Cardiol* 2009; **54**: 595–600.
13. Bossone E, D'Andrea A, D'Alto M, Citro R, Argiento P, Ferrara F, Cittadini A, Rubenfire M, Naeije R. Echocardiography in pulmonary arterial hypertension: from diagnosis to prognosis. *J Am Soc Echocardiogr* 2013; **26**: 1–14.
14. Heinze G, Schemper M. A solution to the problem of monotone likelihood in Cox regression. *Biometrics* 2001; **57**: 114–119.
15. Kaiser R, Grotemeyer K, Lepper P, Stokes C, Bals R, Wilkens H. Associations of circulating natriuretic peptides with haemodynamics in precapillary pulmonary hypertension. *Respir Med* 2015; **109**: 1213–1223.
16. Prins KW, Archer SL, Pritzker M, Rose L, Weir EK, Sharma A, Thenappan T. Interleukin-6 is independently associated with right ventricular function in

pulmonary arterial hypertension. *J Heart Lung Transplant* 2018; **37**: 376–384.
17. Silva Marques J, Martins SR, Calisto C, Goncalves S, Almeida AG, de Sousa JC, Pinto FJ, Diogo AN. An exploratory panel of biomarkers for risk prediction in pulmonary hypertension: emerging role of CT-proET-1. *J Heart Lung Transplant* 2013; **32**: 1214–1221.
18. Nickel N, Jonigk D, Kempf T, Bockmeyer CL, Maegel L, Rische J, Laenger F, Lehmann U, Sauer C, Greer M, Welte T, Hoeper MM, Golpon HA. GDF-15 is abundantly expressed in plexiform lesions in patients with pulmonary arterial hypertension and affects proliferation and apoptosis of pulmonary endothelial cells. *Respir Res* 2011; **12**: 62.
19. Fu S, Ping P, Wang F, Luo L. Synthesis, secretion, function, metabolism and application of natriuretic peptides in heart failure. *J Biol Eng* 2018; **12**: 2.
20. Kutsch J, Faul C, von Scheidt W, Schwaiblmair M, Berghaus TM. The association of the N-terminal pro-brain-type natriuretic peptide response to exercise with disease severity in therapy-naive pulmonary arterial hypertension: a cohort study. *Respir Res* 2018; **19**: 8.
21. Gan CT, McCann GP, Marcus JT, van Wolferen SA, Twisk JW, Boonstra A, Postmus PE, Vonk-Noordegraaf A. NT-proBNP reflects right ventricular structure and function in pulmonary hypertension. *Eur Respir J* 2006; **28**: 1190–1194.
22. Berghaus TM, Kutsch J, Faul C, von Scheidt W, Schwaiblmair M. The association of N-terminal pro-brain-type natriuretic peptide with hemodynamics and functional capacity in therapy-naive precapillary pulmonary hypertension: results from a cohort study. *BMC Pulm Med* 2017; **17**: 167.
23. Helgeson SA, Imam JS, Moss JE, Hodge DO, Burger CD. Comparison of brain natriuretic peptide levels to simultaneously obtained right heart hemodynamics in stable outpatients with pulmonary arterial hypertension. *Diseases* 2018; **6**.
24. Sitbon O, Chin KM, Channick RN, Benza RL, Di Scala L, Gaine S, Ghofrani HA, Lang IM, McLaughlin VV, Preiss R, Rubin LJ, Simonneau G, Tapson VF, Galie N, Hoeper MM. Risk assessment in pulmonary arterial hypertension: insights from the GRIPHON study. *J Heart Lung Transplant* 2020; **39**: 300–309.
25. Burger CD, Long PK, Shah MR, McGoon MD, Miller DP, Romero AJ, Benton WW, Safford RE. Characterization of first-time hospitalizations in patients with newly diagnosed pulmonary arterial hypertension in the REVEAL registry. *Chest* 2014; **146**: 1263–1273.
26. Meoli DF, Su YR, Brittain EL, Robbins IM, Hemnes AR, Monahan K. The transpulmonary ratio of endothelin 1 is elevated in patients with preserved left ventricular ejection fraction and combined pre- and post-capillary pulmonary hypertension. *Pulm Circ.* 2018; **8** 2045893217745019.
27. Wu TY, Majeed A, Kuo KN. An overview of the healthcare system in Taiwan. *London J Prim Care (Abingdon)* 2010; **3**: 115–119.