The prognostic value of various biomarkers in adults with pulmonary hypertension; a multi-biomarker approach

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Background This study aimed to investigate the prognostic value of six different biomarkers in patients with pulmonary hypertension (PH) and to explore whether a multi-biomarker approach can contribute to a better risk stratification.

Methods In this prospective study, patients with PH were included at the day of the diagnostic right heart catheterization between May 2012 and October 2016. Venous blood sampling included; NT-proBNP, high sensitive troponin-T, high sensitive CRP, galectin-3, red blood cell distribution width and eGFR. Associations between biomarker levels and the primary endpoint (death or lung transplantation) and secondary endpoint (death, lung transplantation or heart failure) were assessed with Cox regression, adjusted for age and sex. Additionally, adjustment for the REVEAL risk score was performed.

Results In total, 106 patients were included (median age 58.7 [IQR 47.0-69.2] years, 64% women, 51% pulmonary arterial hypertension). After a median follow-up duration of 23.9 [IQR 15.1-40.0] months, respectively 29 and 37 patients reached the primary and secondary endpoint. All six biomarkers, except eGFR, were significantly associated with the endpoints. A multi-biomarker approach including the number of elevated biomarkers per patient, demonstrated that patients were at higher risk of adverse events as more biomarker levels were elevated (HR for each extra elevated biomarker; 1.33, 95% CI 1.07-1.64, $P = .01$). However, a single as well as a combination of multiple biomarkers, did not yield prognostic value independent of the REVEAL risk score.

Conclusions Various biomarkers are associated with the event-free survival in adults with PH. However, risk stratification exclusively based on single or a combination of biomarkers seems not superior to existing risk scores. (Am Heart J 2019;208:91-9.)

Pulmonary hypertension (PH) is a heterogeneous disease, characterized by an increased pulmonary vascular resistance leading to an elevated pulmonary arterial pressure. Eventually, compensatory mechanisms of the right ventricle may fail to cope with the increased afterload resulting in progressive right-sided heart failure (HF) and death.[1,2] Although treatment options have expanded, morbidity and mortality rates remain high. Risk stratification is crucial to identify patients at high risk and to optimize therapeutic management.

The prognosis of PH varies widely and is besides aetiology,[3] also based on clinical and hemodynamic characteristics including symptoms of HF, 6-minute walking distance, right atrial pressure and cardiac index, according to the European guidelines on PH.[4] Currently, the response to therapy and prognosis is often based on these factors. Biomarkers may provide objective measurements in a relatively non-invasive and easy-accessible manner and the European guidelines on PH advise the use of N-terminal pro B-type natriuretic peptide (NT-proBNP) and troponin-T,[4] which are known to be associated with outcomes in patients with PH.[5,6] Nevertheless, the search for novel biomarkers in PH is ongoing, resulting in new potential biomarkers reflecting various pathophysiological pathways.[7,8] Novel biomarkers, such as galectin-3, but also more common blood biomarkers like red cell distribution width (RDW) and C-reactive protein (CRP), have already been associated with heart failure in different PH groups.[9-12]

Because heterogeneous conditions involving different pathophysiological pathways can give rise to the development and prognosis of PH, multiple biomarkers

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may potentially better reflect a patient's condition. In addition, a combination of biomarkers could provide insight in the main pathophysiological mechanisms within PH subgroups. This study aimed to evaluate diagnosis-specific biomarker profiles and to investigate the associations of these biomarkers with clinical outcomes in patients with PH. Additionally, we explored the prognostic value of a multi-biomarker approach.

Methods

Study design and population

In this prospective observational cohort study, we included all consecutive adult patients with PH at the day of the diagnostic right heart catheterization between the May 2012 and October 2016 in our center. A mean pulmonary artery pressure (mPAP) of ≥25 mmHg measured by right heart catheterization was used as cut-off value for the diagnosis of PH.[4] Exclusion criteria were: incomplete diagnostic work-up and therefore no confirmed PH diagnosis, not treatment-naïve, age <18 years and not capable of understanding and signing informed consent. In addition, we excluded patients with PH due to left heart disease to be able to study biomarker levels caused by primarily right ventricular failure. The study protocol was approved by the medical ethical committee and written informed consent was provided by all patients. This study was performed conform the principles outlined in the Declaration of Helsinki.

World Health Organization classification

Subgroups of PH were classified in accordance with the World Health Organization (WHO) classification of PH[4,13]: pulmonary arterial hypertension (PAH), PH due to lung diseases/hypoxia, chronic thromboembolic pulmonary hypertension (CTEPH) and PH with unclear/multifactorial mechanisms (WHO5). Patients with a mixed clinical picture were grouped under WHO5. Group 1 patients (PAH) were further stratified in subgroups according to the WHO classification.

Data collection

During the inpatient screening visit for analysis of PH, all patients underwent physical examination by a cardiologist and pulmonary physician, 6-minute walking test, spirometry, 12-lead electrocardiography (ECG), echocardiography, venous blood sampling, chest computed tomography scan and right heart catheterization. Patient characteristics and vital signs were collected, including: age, sex, height, weight, blood pressure, heart rate and peripheral oxygen saturation. We used the New York Heart Association (NYHA) functional class to grade the severity of functional limitations by the presence of signs and symptoms of HF.

Biomarker assessment included NT-proBNP, high sensitive troponin-T (hs-TnT), galectin-3, high sensitive CRP (hs-CRP), RDW and eGFR and is further described in Supplementary File 1.

During right heart catheterization, a Swan-Ganz catheter was inserted in the internal jugular vein. A standardized protocol for the work-up of PH was used to obtain hemodynamic measurements and thermodilution or Fick's principle was used to measure the cardiac output. When the obtained capillary wedge pressure was ambiguous, a fluid challenge was performed to distinguish between pre-capillary PH and PH due to left heart disease.

Data was collected and stored in PAHTool (version 4.3.5947.29411, Inovoltus, Santa Maria da Feira, Portugal), an online electronic case report form.

Echocardiography and cardiac computed tomography

Two-dimensional transthoracic echocardiography was performed using a commercially available ultrasound system (iE33, Philips Medical Systems, Best, the Netherlands). For the imaging analysis, we followed the guidelines for cardiac chamber quantification by echocardiography from the American Society of Echocardiography and the European Association of Cardiovascular imaging echocardiography.[14] The systolic left ventricular function was visually graded as normal, mildly, moderately or severely impaired. The presence of pericardial effusion was defined as mild (<10mm), moderate (10-20 mm) or severe (>20mm) in one of the views.

Cardiac computed tomography was performed according to routine clinical practice. The central pulmonary artery diameter and the ascending aortic diameter were measured at the level of the pulmonary artery bifurcation.[15]

Clinical follow-up and definition of endpoints

Patients were prospectively followed-up by half-yearly scheduled visits to the outpatient clinic. Specific PH medications were prescribed when indicated in accordance with the ESC guidelines.[4] Patients with CTEPH eligible for pulmonary endarterectomy or balloon pulmonary angioplasty were referred and treated when indicated. Patients who underwent one of the above procedures, were not censored afterwards.

The primary composite endpoint was defined as all-cause mortality or lung transplantation. The secondary endpoint was a composite of all-cause mortality, lung transplantation or HF-related hospital admission that was defined as any hospitalization due to symptoms or signs of HF requiring (additional) treatment with diuretics.

Survival status of all patients was checked in the Municipal Personal Records database. Suspected endpoint events were adjudicated by two independent researchers based on the electronic patient records. When necessary, we contacted referring hospitals and general practitioners to obtain additional information. Patients who did not reach the primary or secondary endpoint were censored at the 1st of June 2017.

Table I. Baseline characteristics of all patients and stratified according to the subgroups of PH

	Valid cases, n (%)	Total (n = 106)	PAH (n = 54)	PH-lung disease (n = 15)	CTEPH (n = 21)	WHO 5/multifactorial (n = 16)
Clinical characteristics						
Age, years	106 (100)	59[47-69]	55[41-66]	64[55-72]	59[52-73]	64[54-69]
Sex, female n (%)	106 (100)	68 (64)	36 (67)	8 (53)	12 (57)	12 (75)
Body mass index, kg/m^2	106 (100)	28.2±6.5	26.3±5.7	30.5±6.3	31.4±5.7	28.0±8.2
Heart rate, beats/minute	106 (100)	80.1±16.5	79.8±16.2	81.1±12.1	75.2±17.2	86.6±19.5
Systolic blood pressure, mmHg	106 (100)	126.6±17.7	123.1±15.5	125.2±16.6	130.9±13.1	134.2±17.7
Oxygen saturation <90%, n (%)	106 (100)	3 (3)	2 (4)	1 (7)	0 (0)	0 (0)
NYHA class 3/4, n (%)	106 (100)	58 (55)	32 (60)	8 (53)	9 (43)	9 (56)
Electrocardiography						
Rhythm, n (%)	103 (97)					
-Sinus rhythm		92 (89)	47 (89)	13 (87)	18 (95)	14 (88)
-Atrial fibrillation		7 (7)	4 (7)	2 (13)	0 (0)	1 (6)
-Other		4 (4)	2 (4)	0 (0)	1 (5)	1 (6)
QRS duration, ms	102 (96)	98[90-106]	100[91-106]	100[91-111]	94[88-99]	99[88-114]
6-minute walking test						
Distance, m	90 (85)	338.1±139.1	347.7±147.3	309.0±115.4	685.1±129.8	273.5±126.4
Echocardiography						
RA area, cm^2	81 (76)	27.3±8.9	27.8±6.9	28.4±8.4	24.6±10.0	27.4±13.0
RV basal dimension, mm	76 (72)	51.3±9.5	52.5±8.1	47.8±4.7	51.1±12.1	49.8±12.6
RV fractional area change, %	74 (70)	29.1±8.6	27.0±8.0	31.3±6.2	33.0±4.3	30.8±12.2
RV TAPSE, mm	73 (69)	19.4±4.9	18.6±4.8	18.8±3.1	20.9±3.5	20.4±7.0
LV function, n (%):	100 (94)					
-Normal		66 (66)	33 (63)	10 (71)	15 (83)	8 (50)
-Mildly impaired		30 (30)	17 (33)	4 (29)	3 (17)	6 (38)
-Moderately impaired		3 (3)	2 (4)	0 (0.0)	0 (0.0)	1 (6)
-Severely impaired		1 (1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6)
LV end diastolic dimension, mm	82 (77)	43.4±7.5	41.0±7.2	46.1±7.3	45.5±5.6	47.0±8.1
Right heart catheterization						
mPAP, mmHg	106 (100)	42.0[34.8-51.3]	49.2[38.0-59.0]	37.0[32.0-41.0]	37.0 [30.0-48.0]	42.5[34.0-48.5]
mRAP, mmHg	106 (100)	9.9±5.4	10.6±5.6	8.3±3.8	7.8±4.7	11.7±5.4
Capillary wedge pressure, mmHg	92 (87)	13.2±6.2	11.3±5.6	12.5±4.3	13.7±3.3	18.7±8.2
Cardiac output, L/min	101 (95)	5[4.0-6.3]	4.9[3.9-5.7]	5.0[3.9-5.8]	5.4[4.9-6.3]	5.1[4.0-6.8]
Cardiac index, L/min/m^2	101 (95)	2.6[2.2-3.3]	2.53[2.17-3.55]	2.57[2.09-2.91]	2.7[2.3-3.0]	2.7 [2.1-3.6]
PVR, Wood units	89 (84)	5.5[3.3-9.3]	7.1[5.1-11.8]	4.4[4.1-5.5]	3.4[3.0-5.]	4.7 [2.3-6.8]
Computed tomography						
PA diameter, mm	100 (94)	34.4±5.3	35.3±6.0	34.9±4.1	34.3±5.1	31.1±3.2
PA/AO ratio	100 (94)	1.12±0.23	1.20±0.26	1.03±0.12	1.12±0.24	0.97±0.12

Legend: Values are represented as mean±SD or median[IQR].
Abbreviations: RA, right atrial; RV, right ventricular; LV, left ventricular; mRAP, mean right arterial pressure; PA, pulmonary artery; AO, aortic artery.

Statistical analysis

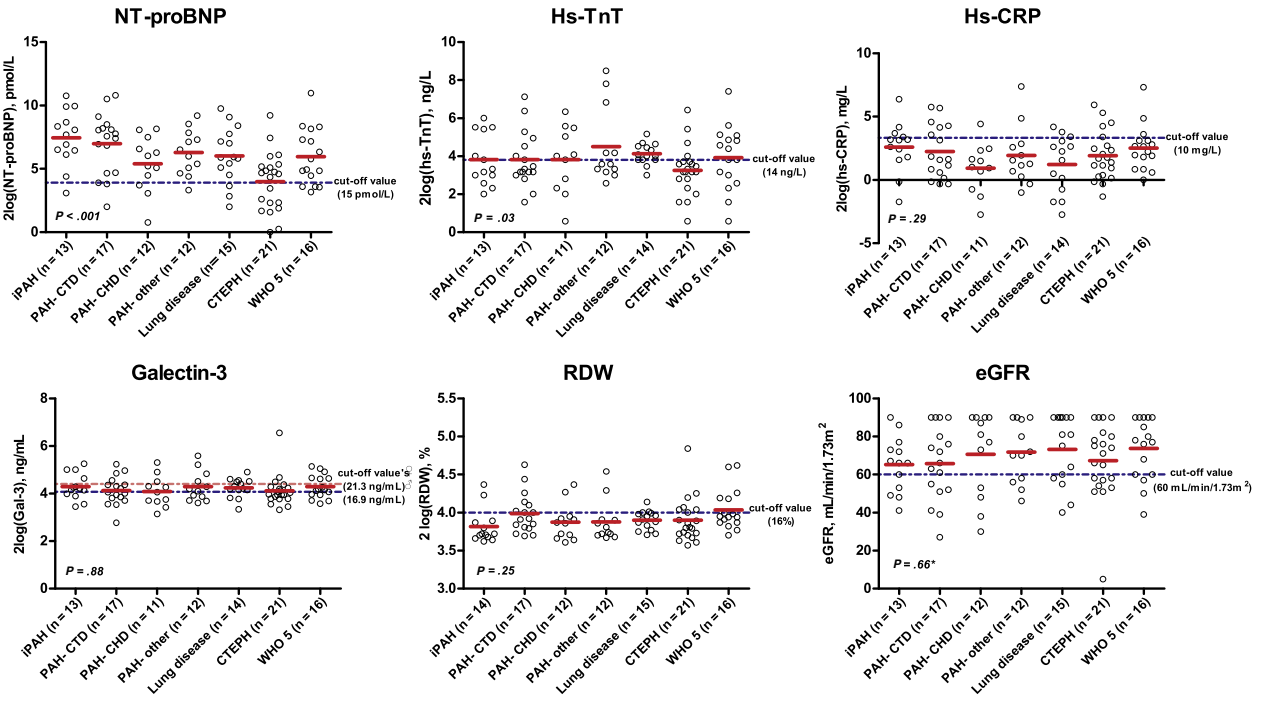
Continuous variables were presented as mean ± standard deviation (SD) or median [interquartile range]. Biomarker levels were 2-log transformed to correct for skewness. Biomarker values below the limit of detection (LoD) were substituted with a value that was equal to 50% of the LoD, for analytical purposes.

Biomarker release was visualized in scatterplots. Comparisons of biomarker levels across PH subgroups were performed using a one-way ANOVA test or Kruskal-Wallis test. Correlations were reflected by the Pearson or Spearman correlation coefficient, depending on the distribution of the data. Tertiles of the biomarker distributions were determined. The cumulative survival for the tertiles was calculated using the Kaplan-Meier estimator; comparisons between survival tertiles were made using the log-rank test for trend.

To compare the effect sizes of the different biomarkers, biomarker levels were standardized according to the mean and SD of their distribution, and the relation between the thus obtained Z-scores and study endpoints were evaluated by Cox proportional-hazards regression. Multivariable analysis was performed to adjust for age and sex. The corresponding C-index, reflecting the discriminative ability of each biomarker up to 40 months, was calculated.

Subsequently, all patients with complete biomarker profiles were included in a multi-biomarker model. Biomarkers were classified as normal or elevated according to the pre-specified cut-off value. The association of the number of elevated biomarkers with the study endpoints was evaluated by Cox regression. As post-hoc analysis, we determined for each patient the REVEAL risk score based on the full PAH risk calculator.[16] We defined renal insufficiency as a eGFR <30mL/min per 1.73 m^2. Missing data of the

Figure 1



Biomarker levels stratified according to the WHO classification of PH. Legend: Biomarker levels are represented on the 2-log scale (except for eGFR). Blue dotted line represents the biomarker-specific cut-off value. Red line represents the mean biomarker level in each group. For galectin-3, sex-specific biomarker cut-off values are represented (blue dotted line= man, pink dotted line= woman). * Kruskal Wallis test, otherwise one-way ANOVA test.

parameters was taken care of with multiple imputation, with 5 imputations. We adjusted every biomarker for the REVEAL score in separate analyses.

Statistical analysis was performed using IBM SPSS software (version 21.0.0.1). The C-index was calculated in R (version 3.3.3), packages SurvC1 and Survival. A 2-sided P value < .05 was considered statistically significant.

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Results

Baseline characteristics

A total of 164 consecutive patients underwent right heart catheterization as part of the screening for PH between 15th of May 2012 and 4th of October 2016, of which 106 patients fulfilled the inclusion criteria and were enrolled in the study (Supplementary File 2). Patients were classified as PAH (n = 54, 51%), PH-lung disease (n = 15, 14%), CTEPH (n = 21, 20%) and WHO5/ multifactorial (n = 16, 15%). Baseline characteristics of all patients and specified for each PH diagnosis, are summarized in Table I. Median age was 58.7 [IQR 47.0-69.2] years, 68 (64%) were woman and 58 (55%) were in NYHA class 3 or 4. In 8 patients pericardial effusion was present, of which 7 mild and one moderate.

Biomarker levels according to the PH classification

Baseline levels of the six biomarkers stratified according to subgroups of PH are shown in Figure 1. Because of small numbers, heritable PAH, PAH induced by drugs and toxins, PAH associated with portal hypertension and PAH caused by pulmonary veno-occlusive disease were grouped as 'other'.

NT-proBNP significantly differed between the PH subgroups (p<0.001). The highest levels of NT-proBNP were found in the patients with iPAH (median 205.0 pmol/L, IQR 87.9-407.0 pmol/L) and PAH associated with connective tissue disease (median 190.0 pmol/L, IQR 25.9-293.0 pmol/L). NT-proBNP was elevated in 82 (77%) patients. Of the patients with iPAH, all except one had an elevated NT-proBNP. Hs-TnT also differed significantly between the PH subgroups ($P = .03$) and levels were lowest in CTEPH patients (median 9.0 ng/L, IQR 7.0-13.0 ng/L). In 43 patients (41%), an elevated level of hs-TnT was

Table II. Correlation coefficients of biomarkers with baseline characteristics

	NT-proBNP	Hs-TnT	Hs-CRP	Galectin-3	RDW	eGFR†
	n = 106	n = 104	n = 104	n = 104	n = 106	n = 106
	r	*r*	*r*	*r*	*r*	*r*
Clinical characteristics						
Age, years†	0.10	**0.36*****	-0.19	**0.28****	0.15	**-0.43*****
Sex, female	0.05	**0.25***	-0.11	-0.01	0.03	0.01
Body mass index, kg/m^2	-0.15	-0.04	**0.24***	0.13	-0.01	-0.06
Heart rate, beats/minute	0.15	0.08	0.19	**0.26****	0.13	-0.01
Systolic blood pressure, mmHg	**-0.22***	-0.18	-0.04	-0.13	**-0.21***	0.04
Oxygen saturation <90%	-0.02	-0.06	-0.07	-0.02	0.07	0.13
NYHA class 3/4	**0.40*****	0.18	**0.23***	**0.30****	0.19	**-0.38*****
Electrocardiography						
Loss of sinus rhythm	0.11	0.06	-0.13	-0.08	-0.05	0.01
QRS duration, ms†	0.11	**0.24***	-0.14	0.05	0.07	-0.01
6-minute walking test						
Distance, m	**-0.44*****	**-0.38****	**-0.32****	**-0.44*****	**-0.33****	**0.40*****
Echocardiography						
RA area, cm^2	**0.41*****	0.17	-0.13	-0.03	-0.01	**-0.25***
RV basal dimension, mm	**0.49*****	0.18	0.13	0.03	-0.02	**-0.28***
RV fractional area change, %	**0.34****	-0.17	-0.13	-0.19	-0.05	0.19
RV TAPSE, mm	**0.43*****	-0.19	-0.15	0.00	-0.06	-0.01
LV function, 0-3	**0.35*****	0.14	0.14	0.05	0.19	-0.08
LV end diastolic dimension, mm	**-0.31****	-0.12	-0.16	-0.10	0.02	**0.39*****
Right heart catheterization						
mPAP, mmHg†	**0.42*****	0.03	0.10	-0.01	0.07	-0.07
mRAP, mmHg	**0.30****	0.16	0.18	0.16	0.13	-0.18
Capillary wedge pressure, mmHg	-0.06	-0.03	0.01	0.07	0.03	0.02
Cardiac output, L/min†	**0.46*****	-0.17	-0.06	-0.01	0.03	0.18
Computed tomography						
PA diameter, mm	0.03	0.11	-0.14	-0.05	-0.08	-0.03
PA/AO ratio	-0.10	-0.13	-0.13	**-0.26****	-0.15	0.16

Legend: Level of significance is indicated by the number of asterisks. *indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001. †Spearman, otherwise Pearson correlation coefficient. Significant values are presented in bold.
Abbreviations: RA, right atrial; RV, right ventricular; LV, left ventricular; mRAP, mean right arterial pressure; PA, pulmonary artery; AO, aortic artery.

measured. We found no significant differences in the levels of galectin-3, hs-CRP, RDW and eGFR between the subgroups of PH.

Association between biomarkers and baseline characteristics

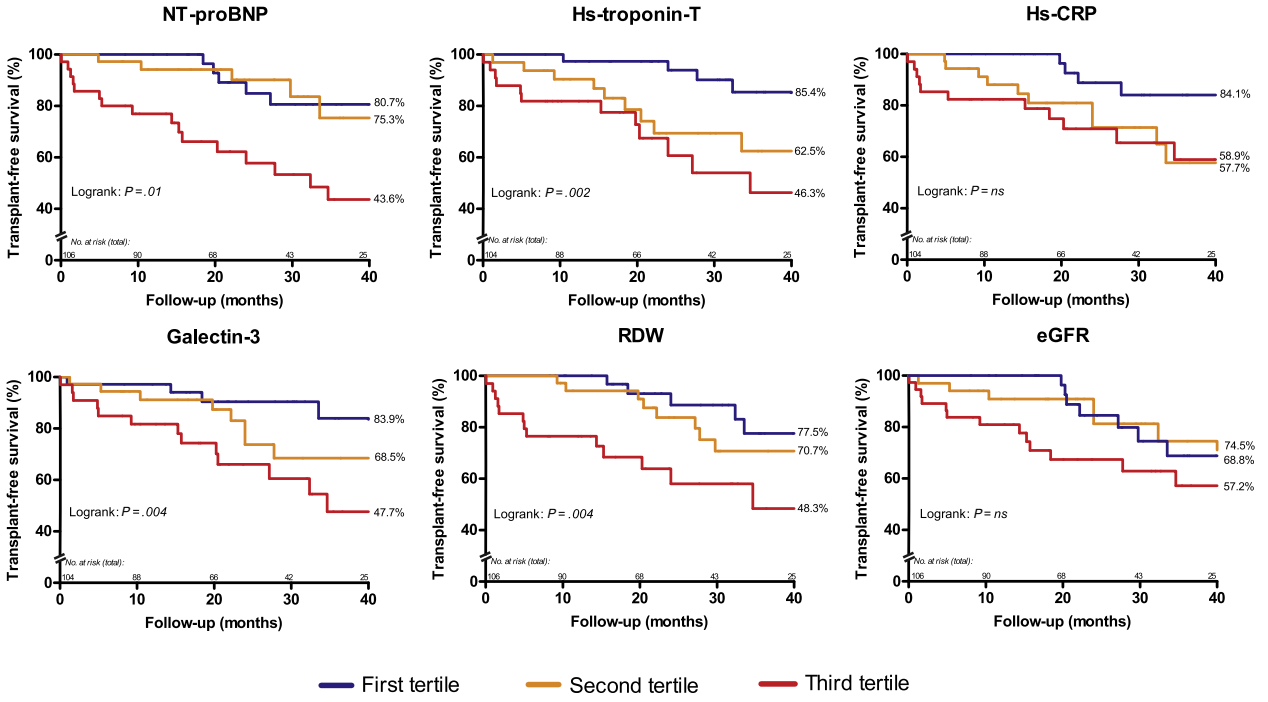
As shown in Table II, all biomarkers significantly correlated with the 6-minute walking distance, and all except hs-TnT and RDW showed a significant correlation with NYHA class. NT-proBNP showed the strongest correlation with both the 6-minute walking distance (r = -0.46, $P < .001$) and NYHA class (r = 0.40, $P < .001$). NT-proBNP correlated with right ventricular and left ventricular function echocardiographic variables. In addition to NT-proBNP, only eGFR correlated with right atrial area, right ventricular basal dimension and left ventricular end diastolic dimensions. Hemodynamic measurements exclusively showed a significant correlation with NT-proBNP levels. Mutual correlations between biomarkers were all significant. The strongest correlation was found between hs-TnT and galectin-3 (r = 0.58, $P < .001$) (Supplementary File 3).

Follow-up

The median follow-up duration was 23.9 (IQR 15.1-40.0) months. Follow-up data regarding mortality and other endpoints was 100% complete. During follow-up 25 patients died and 4 patients underwent lung transplantation, so the primary endpoint was reached in 29 patients (27.4%). Causes of death included end-stage HF (n = 8), sudden death, presumed cardiac (n = 4), euthanasia in patients with end-stage cardiovascular and pulmonary disease (n = 3), multi-organ failure (n = 3), kidney and/or liver failure (n = 2), myocardial infarction (n = 1), progression of systemic sclerosis (n = 1), hepatic encephalopathy (n = 1), malignancy (n = 1) and sudden death, presumed cerebral (n = 1).

Twenty-two patients were hospitalized for HF requiring (additional) diuretic treatment. This resulted in 37 (34.9%) patients who reached the secondary endpoint. Of the patients with PAH, 49 (91%) patients used any PH medication during follow-up (20% monotherapy, 33% duo therapy, 37% triple therapy). Considering the patients with CTEPH, three patients underwent balloon

Figure 2



Transplant-free survival according to tertiles of the biomarker levels. Legend: NT-proBNP: first tertile <26.57 pmol/L, second tertile 26.57-150.0 pmol/L, third tertile >150.0 pmol/L. **Hs-TnT**: first tertile <2.0 ng/L, second tertile 2.0-6.3 ng/L, third tertile >6.3 ng/L. **Hs-CRP**: first tertile <2.0 mg/L, second tertile 2.0-6.3 mg/L, third tertile >6.3 mg/L. **Galectin-3**: first tertile ≤15.3 ng/mL, second tertile 15.3-19.9 ng/mL, third tertile >19.9 ng/mL. **RDW**: first tertile ≤ 13.6%, second tertile 13.6-15.5%, third tertile >15.5%. **eGFR**: first tertile >80.3 ml/min, second tertile 60.0-80.3 ml/min, third tertile ≤60 ml/min (Tertiles of eGFR are inversed).

pulmonary angioplasty, three patients underwent pulmonary endarterectomy surgery and 15 patients used PH medication.

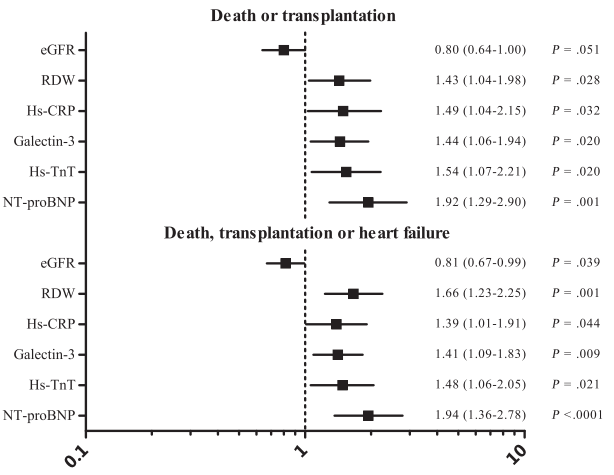
Associations between biomarkers and clinical outcomes

The transplant-free survival according to tertiles of biomarker levels are visualized in the Kaplan-Meier curves (Figure 2). Patients in the highest tertiles of NT-proBNP, hs-TnT, galectin-3 and RDW had a significantly higher risk of death or transplantation compared to patients in the lower tertiles.

Concerning the secondary endpoint, patients in the highest tertiles of NT-proBNP, hs-TnT, galectin-3 and RDW, were at highest risk of death, transplantation or HF. For eGFR, patients in the highest tertile of eGFR had a significant better event-free survival than patients in the lower tertiles. (Supplementary File 4)

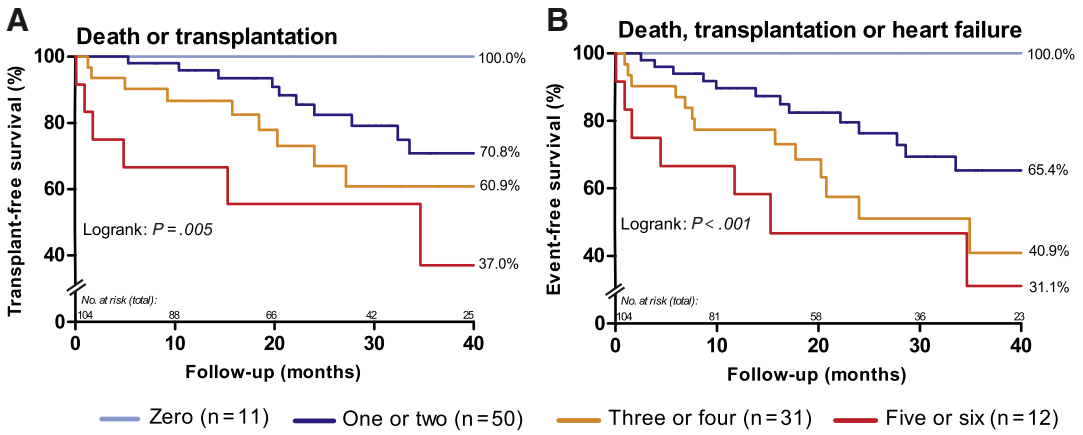
Standardized hazard ratios for the association between biomarker levels and the endpoints are shown (Figure 3). When analyzed continuously, all biomarkers except eGFR, showed a significant association with the primary endpoint, NT-proBNP, hs-CRP and hs-TnT had the highest hazard ratios. According to the C-index, NT-proBNP had the best discriminative power (C-index 0.69, 95% CI 0.58-

Figure 3



Standardized hazard ratios, reflecting the instantaneous risk of the primary and secondary endpoint per one standard deviation increase in the 2-log-transformed biomarker level Legend: Standardized hazard ratios adjusted for age and sex, with the corresponding 95% CI and p-value for each biomarker.

Figure 4



Transplant-free and event-free survival stratified according to the number of elevated biomarkers. Legend: Figure legend shows the number of elevated biomarkers with the corresponding line color. *for eGFR lowered levels were taken into account. (detailed explanation of the different groups is provided in Supplementary File 7).

0.81), followed by hs-TnT (C-index 0.67, 95% CI 0.55-0.78) and galectin-3 (C-index 0.67, 95% CI 0.52-0.83) (Supplementary File 5). All biomarkers were significantly associated with the secondary endpoint. For RDW, the standardized hazard ratio of the secondary endpoint increased relatively to the hazard ratio of the primary endpoint, as did the C-index.

When biomarker levels were adjusted for the REVEAL risk score, none of the biomarkers remained significantly associated with the endpoints. (Supplementary File 6)

Multi-biomarker approach

Figure 4 shows the Kaplan-Meier curve stratified according to the number of elevated biomarkers. Patients with zero elevated biomarkers were free of events after 40 months whereas of the patients with five or six elevated biomarkers, only 37% was alive and free of transplantation. The age and sex adjusted hazard ratio for the primary endpoint for one extra elevated biomarker was 1.34 (95% CI 1.08-1.67, P = .009). Multi-biomarker analysis concerning the secondary endpoint (Figure 4B) showed similar results (HR 1.33, 95% CI 1.09-1.61, P = .004). However, when adjusted for the REVEAL risk score, the number of elevated biomarkers did not yield any independent prognostic value for both endpoints (HR 1.06, 95% CI 0.83-1.36, P =.63 and HR 1.09, 95% CI 0.88-1.57, P =.44 respectively).

Discussion

This study investigated diagnosis-specific biomarker profiles, the prognostic value of these biomarkers and the potential benefit of a multi-biomarker approach in adult patients with PH. A significant difference in the biomarker levels of NT-proBNP and hs-TnT among the different subgroups of PH was found and only 11% of the patients were free of any abnormal biomarker level. All six biomarkers studied in this prospective cohort were associated with adverse clinical outcomes, independent of age and sex. A multi-biomarker approach demonstrated that patients were at higher risk of events as more biomarkers were elevated, wherein the absence of any elevated biomarker ruled out the risk of any event up to 40 months. However, the additive value of individual biomarkers as well as a multi-biomarker model, compared to the existing REVEAL risk score, seems limited. This emphasizes that adequate risk stratification in PH may only be achieved by incorporating multiple clinical characteristics and modalities into a comprehensive risk model.

Strengths of this study are its prospective design and the inclusion of only treatment naïve patients. The biomarkers therefore reflect a more natural state of disease severity, which is unaffected by treatment status. Additionally, this study evaluated the levels of six biomarkers measured at one point in time reflecting a patients underlying disease state at a single moment. Patients with PH due to left heart disease were excluded in this study to prevent any interference in biomarker levels due to myocardial stress primary caused by the left ventricle. We aimed to base our conclusions merely on processes regarding failure of the right ventricle.

New biomarker for PH: galectin-3

To our knowledge, this is the first prospective cohort study investigating the association with galectin-3 and clinical outcomes in patients with heterogeneous types of pre-capillary PH. Galectin-3 belongs to the beta-galactose-binding lectins and induces cardiac fibroblast proliferation,

a process associated with the development of HF.[17] Galectin-3 may particularly be appropriate to monitor right ventricular remodelling.[18] Previous studies investigating galectin-3 mainly focused on patients with PAH only and consisted of smaller cohorts.[9,18,19] Calvier et al found elevated levels of galectin-3 in PAH patients, which correlated with NYHA class.[19] Mazurek et al demonstrated that galectin-3 was associated with mortality, however this study included both patients with PAH and patients with PH due to left heart disease.[9] In our current study, we had the unique opportunity to compare different subgroups of PH. Only 38.5% of the patients with a galectin-3 level of >19.9 ng/mL was alive and free of transplantation or HF after 40 months. Galectin-3 should therefore be considered as a new potential prognostic biomarker in the pre-capillary PH population.

Cardiovascular health 'barometer': red blood cell distribution width

Previous studies investigating the prognostic role of RDW in patients with PH already demonstrated that levels of RDW are associated with mortality,[11] even independent of NT-proBNP.[10] A variety of mechanisms have been hypothesized to elucidate the prognostic value of RDW in HF patients. Levels of RDW appear to reflect an underlying inflammatory state and an impaired iron metabolism. It has been suggested that RDW can be seen as an overall cardiovascular health 'barometer'.[20,21]

In our study, elevated levels of RDW were observed in all subgroups of PH and were associated with death or transplantation, independent of age and sex. Patients with RDW >15%, have a cumulative transplant-free survival of only 48.3% after 40 months. Adding HF-related hospital admission to the endpoints increased the hazard ratio as well as the C-index. This suggests that besides being able to predict mortality or transplantation, RDW may be even better in predicting HF. Although it did not yield any independent prognostic value besides the REVEAL risk score, it would be interesting to know whether incorporation of RDW in the risk score can improve risk stratification and if it can be a good predictor for heart failure. Specifically since RDW is determined as part of the automated blood count and is therefore inexpensive, easy-accessible and widely available as biomarker.

Heterogeneity across the PH population

A difficulty when studying the PH population is the major diversity in etiology and the presence of PH-related comorbidities. The baseline characteristics of our study showed differences in disease severity among the PH subgroups, it is therefore likely that the prognostic value of the biomarkers in these subgroups also differ. The prognosis may be explained by the interaction between PH and the PH-related comorbidities, which differs between individuals. Cause of death in our study was diverse, with only 8 patients dying merely due to end-stage HF. It is therefore not surprising that many (potential) biomarkers are suggested for the risk stratification and that there is an increasing demand for a multi-biomarker approach that can capture more pathophysiological axes.[7] Indeed, the different biomarkers in this study demonstrate that the pathophysiology of PH is complex and that one should not merely focus on one all-encompassing biomarker.

Future perspectives

This study shows that NT-proBNP yields the strongest prognostic and discriminative value for adverse cardiac events in adults with PH. NT-proBNP is already incorporated in the REVEAL risk score and PH guidelines. The change of the REVEAL risk score over time has also been investigated,[22] however the REVEAL risk score is comprehensive and consists of many invasive as well as less invasive measurements. Invasive hemodynamic measurements may be inconvenient to measure frequently, it would therefore be interesting if biomarkers could be a surrogate marker for one of these variables. The multi-biomarker approach is just a simple reflection of how multiple biomarker can contribute to the risk stratification in PH and should be further investigated in larger studies with the use of continuous levels in a multivariable analysis. Additionally, it would be of interest to investigate whether serial biomarker measurements may improve prognostic precision and can monitor therapy.

Study limitations

NT-proBNP, RDW and eGFR were directly determined in the clinical laboratory and therefore these biomarker results were directly available for the treating physician of a patient. Particularly NT-proBNP is an established biomarker and is used for the risk assessment in PH.[4] It was therefore inescapable and not ethical, to ignore NT-proBNP in the considerations of the clinical management. As a result, this may have diluted the association between NT-proBNP and clinical outcomes.

During the study, patients were treated goal oriented and in accordance with the ESC guidelines. Due to the long inclusion period, treatment strategies may have changed over time specifically regarding the introduction of combination therapy in PAH patients.[23] This may have introduced differences in prognosis in our study. Six patients with CTEPH underwent pulmonary endarterectomy or balloon pulmonary angioplasty. Patients were not censored after the procedure because this would have introduced bias. However, keeping this patients in the study may also have biased the association between biomarker levels and clinical events, most likely towards the null.

Conclusions regarding the multi-biomarker approach should be treated with caution. This study demonstrates an association between the number of elevated biomarkers and the risk of events.

However, we could not correct for the underlying biomarker correlations in a multivariable-analysis due to limited numbers of events. The biomarkers may share some common prognostic effect; hence the exact additive prognostic value of each biomarker is difficult to compute. Also, the number of elevated biomarkers is analyzed continuously instead of categorical due to limited degrees of freedom.

Due to the relatively small sample size and limited number of events, the power of this study was not sufficient to correct for the different PH subgroups.

Conclusions

This study showed that a wide range of biomarkers reflecting different pathophysiological pathways are significantly associated with an increased risk of mortality, lung transplantation or HF in adults with PH. However, the additive value of a single biomarker compared to already existing risk scores, is limited. Combining multiple biomarkers may be beneficial in detecting patients at higher risk of events, nevertheless risk stratification based on exclusively biomarkers seems inadequate.

Appendix. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ahj.2018.11.001.

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