The novel marker LTBP2 predicts all-cause and pulmonary death in patients with acute dyspnoea

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ABSTRACT

The risk stratification in patients presenting with acute dyspnoea remains a challenge. We therefore conducted a prospective, observational cohort study enrolling 292 patients presenting to the emergency department with acute dyspnoea. A proteomic approach for antibody-free targeted protein quantification based on high-end MS was used to measure LTBP2 [latent TGF (transforming growth factor)-binding protein 2] levels. Final diagnosis and death during follow-up were adjudicated blinded to LTBP2 levels. AHF (acute heart failure) was the final diagnosis in 54% of patients. In both AHF ($P < 0.001$) and non-AHF ($P = 0.015$) patients, LTBP2 levels at presentation were significantly higher in non-survivors compared with survivors with differences on median levels being 2.2- and 1.5-fold respectively. When assessing the cause of death, LTBP2 levels were significantly higher in patients dying from pulmonary causes ($P = 0.0005$). Overall, LTBP2 powerfully predicted early pulmonary death {AUC (area under the curve), 0.95 [95% CI (confidence interval), 0.91–0.98]}. In ROC (receiver operating characteristic) curve analyses for the prediction of 1-year mortality LTBP2 achieved an AUC of 0.77 (95% CI, 0.71–0.84); comparable with the predictive potential of NT-proBNP [N-terminal pro-B-type natriuruetic peptide; 0.77 (95% CI, 0.72–0.82)]. Importantly, the predictive potential of LTBP2 persisted in patients with AHF as the cause of dypnea (AUC 0.78) and was independent of renal dysfunction (AUC 0.77). In a multivariate Cox regression analysis, LTBP2 was the strongest independent predictor of death [HR (hazard ratio), 3.76 (95% CI, 2.13–6.64); $P < 0.0001$]. In conclusion, plasma levels of LTBP2 present a novel and powerful predictor of all-cause mortality, and particularly pulmonary death. Cause-specific prediction of death would enable targeted prevention, e.g. with pre-emptive antibiotic therapy.

Key words: acute dyspnoea, diagnosis, latent transforming-growth-factor-binding protein (LTBP), prognosis, pulmonary death.

Abbreviations: AHF, acute heart failure; AUC, area under the curve; BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CT, computed tomography; CV, coefficient of variation; ECM, extracellular matrix; FFE, free-flow electrophoresis; GFR, glomerular filtration rate; eGFR, estimated GFR; HR, hazard ratio; IQR, interquartile range; NP, natriuretic peptide; BNP, B-type NP; NT-proBNP, N-terminal proBNP; NYHA, New York Heart Association; ROC, receiver operating characteristic; SRM, selected reaction monitoring; TGF, transforming growth factor; LTBP, latent TGF-binding protein.

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INTRODUCTION

Acute dyspnoea is one of the most common chief complaints of patients presenting to the emergency department [1], with the array of possible underlying pathologies ranging from anxiety and hyperventilation to life-threatening pulmonary, cardiac and metabolic causes. Unfortunately, the signs and symptoms of these various diseases are neither specific nor sensitive, making rapid and accurate triage and risk-stratification difficult.

NPs (natriuretic peptides) as quantitative biomarkers of cardiac haemodynamic stress have revolutionized the early diagnosis and risk stratification of dyspneic patients by accurately identifying patients experiencing increased cardiac stress [2–4]. However, biomarkers identifying pulmonary stress and accurately detecting patients at highest risk of pulmonary complications are currently still missing.

LTBP2 [latent TGF (transforming growth factor)-binding protein 2] was recently discovered in an unbiased proteomics search for novel markers in patients with acute dyspnoea [5]. LTBP2 is an ECM (extracellular matrix) protein with multiple functions ranging from controlling TGFβ activity to ECM structure and cell adhesion [6]. Importantly, the highest levels of LTBP2 are consistently found in lung tissue [7]. We therefore aimed to assess the diagnostic and prognostic potential of LTBP2 in patients presenting with acute dyspnoea.

MATERIALS AND METHODS

Study population

The study population consisted of unselected patients presenting to the emergency department of the University Hospital of Basel, Switzerland, with a chief complaint of acute dyspnoea. From April 2006 to March 2007, 292 patients (out of 327 patients screened) were prospectively enrolled. Exclusion criteria were age younger than 18 years, an obvious traumatic cause of dyspnoea and patients on haemodialysis. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committee (Reference Number EK 52/06). Written informed consent was obtained from all participating patients.

Clinical evaluation and follow-up

Patients underwent an initial clinical assessment including clinical history, physical examination, ECG, pulse oximetry, blood tests including BNP (B-type NP) and chest X-ray. Echocardiography, pulmonary function tests and other diagnostic tests such as CT (computed tomography) angiography were performed according to the decisions of the treating physician. CT angiography was the imaging modality of choice in patients with suspected pulmonary embolism. To assess the dyspnoea severity, we used the NYHA (New York Heart Association) functional classification with NYHA II as 'dyspnoea while walking up a slight incline', III as 'dyspnoea while walking on level ground' and IV as 'dyspnoea at rest'.

Two independent internists blinded to LTBP2 reviewed all medical records including BNP levels and independently classified the patients' primary diagnosis into seven categories: AHF (acute heart failure), acute exacerbation of COPD (chronic obstructive pulmonary disease), community acquired pneumonia, acute complications of malignancy, acute pulmonary embolism, hyperventilation, and others. The two internists also independently adjudicated the cause of death. In the event of diagnostic disagreement among the internist reviewers, they were asked to meet to come to a common conclusion. In the event that they were unable to come to a common conclusion, a third-party internist adjudicator was asked to review the data and determine which diagnosis and cause of death was the most accurate.

The end point of the present study was 30-day cause specific mortality. 30-day all-cause mortality, 1-year cause-specific mortality and 1-year all-cause mortality were assessed as secondary end points. Cardiac death was defined as death due to coronary artery disease, heart failure or arrhythmias. Pulmonary death was defined as death due to acute exacerbations of COPD, pneumonia and asthma. Each patient was contacted for follow-up, via telephone, by a single trained researcher after 365 days. In case the patient could not be reached, referring physicians and relatives were contacted or the administrative databases of respective hometowns were reviewed to assess the survival status. Of note, one patient was lost to follow-up, so 1-year mortality analyses were performed in 291 patients.

Laboratory measurements

Blood samples for determination of LTBP2, BNP and NT-proBNP (N-terminal pro-BNP) were collected at presentation into tubes containing potassium EDTA. After centrifugation, samples were frozen at $-80\,^{\circ}C$ until assayed in a blinded fashion in a single batch. NT-proBNP levels were determined by a blinded fashion by a quantitative electrochemiluminescence immunoassay with CVs (coefficients of variation) reported by the manufacturer of 1.8–2.7% and 2.35–3.2% for within-run and total imprecision respectively (Elecsys proBNP; Roche Diagnostics) [8] and BNP was measured by a microparticle enzyme immunoassay at the hospital laboratory with CVs reported by the manufacturer of 4.3–6.3% and 6.5–9.4% for within-run and total imprecision, respectively (AxSym; Abbott Laboratories) [9]. LTBP2 levels were measured using targeted MS based on an SRM (selected reaction monitoring) peptide quantification method [10]. In short, blood samples were depleted from albumin and IgG and then

digested into peptides using trypsin. One such peptide, unique for LTBP2 in the human proteome (EQDAPVAGLQPVER), was then quantified against a known amount of its isotopically labelled variant spiked in each sample. Reverse-phase chromatography in nano-mode was used to separate the peptide mixtures (Ultimate 3000; Dionex) before quantification using a triple quadrupole MS instrument (Vantage TSQ; Thermo Scientific) operated in single-reaction-monitoring mode. To reach the desired sensitivity, an upfront peptide fractionation step based on the specific isoelectric properties of the peptide of interest was incorporated in the process using FFE (free-flow electrophoresis; BD Diagnostics). FFE fractions containing the peptide of interest were pooled and analysed using SRM analysis. The read-out of this assay in a sample is defined as the ratio of the analyte peak and the common spiked internal standard peptide peak. For each sample analysed a ratio was obtained and comparison of ratios between different samples represents the relative quantification of the protein. These SRM-based protein quantifications provide valuable tools in the measurement of proteins for which immunoassays are not yet available.

Statistical analysis

Continuous variables are presented as means $\pm$ S.D. or medians [IQR (interquartile range)], and categorical variables as numbers and percentages. Univariate data on demographic and clinical features were compared by Mann–Whitney *U* test or Fisher's exact test as appropriate. Correlations among continuous variables were assessed by the Spearman rank-correlation coefficient. ROC (receiver operating characteristic) curves were utilized to evaluate the accuracy of LTBP2, NT-proBNP and BNP to predict death. AUCs (areas under the curve) were calculated for all markers. AUCs were compared according to the method by Hanley and McNeil [11]. Survival analyses were performed using Cox regression analysis and Kaplan–Meier survival curves. The Kaplan–Meier cumulative survival curves were compared by the log-rank test. Cox regression analysis was assessed by univariate and multivariate analysis to identify independent predictors of outcome. Overall 38 clinical and laboratory candidate variables were assessed in univariate regression analysis. Multivariable analysis included all significant candidate variables ($P < 0.05$) established in univariate analysis. GFR (glomerular filtration rate) was calculated using the abbreviated MDRD (modification of diet in renal disease) formula. Data were analysed statistically with SPSS 19.0 software and the MedCalc 9.3.9.0 package. All probabilities were two-tailed, and $P < 0.05$ was regarded as significant. Provided *P* values are not adjusted for multiple comparisons.

RESULTS

Patient characteristics

The baseline characteristics of the 292 patients presenting with acute dyspnoea are described in Table 1. Overall, mean age was 74 ± 12 years [median (IQR), 77 (68–83) years), 52 % were men and 80 % were in NYHA functional classes III and IV. The primary diagnosis was AHF in 158 (54 %) patients, acute exacerbation of COPD in 57 (20 %) patients, pneumonia in 33 (11 %) patients, acute pulmonary embolism in eight (3 %) patients, acute complications of malignancy in seven (2 %) patients, hyperventilation in five (2 %) patients, and other causes such as interstitial lung disease, asthma, or bronchitis in 24 (8 %) patients.

LTBP2 levels as a function of HF diagnosis

Median levels of LTBP2 increased steadily with increasing cardiac stress across the final diagnoses (Figure 1A). A similar trend was observed for NT-proBNP (N-terminal proBNP) levels (Figure 1B). Concentrations of LTBP2 were twice as high in those patients with dyspnoea judged to have AHF [0.017 (0.010–0.033) normalized level in AHF compared with 0.008 (0.004–0.014] normalized level in non-AHF; $P < 0.001$]. On the basis of ROC analyses, LTBP2 had an AUC of 0.75 for the diagnosis of AHF [95 % CI (confidence interval), 0.69–0.81; $P < 0.001$), whereas NT-proBNP and BNP had AUCs of 0.92 (95 % CI, 0.88–0.95) and 0.94 (95 % CI, 0.92–0.97) respectively].

LTBP2 concentrations at presentation in patients with dyspnoea were strongly correlated with age ($r = 0.59$; $P < 0.001$), markers of kidney dysfunction (creatinine: $r = 0.71$, $P < 0.001$; cystatin C: $r = 0.83$, $P < 0.001$, haemoglobin: $r = -0.38$, $P < 0.001$ potassium: $r = 0.33$; $P < 0.001$), BNP ($r = 0.52$, $P < 0.001$) and NT-proBNP ($r = 0.66$, $P < 0.001$). Consequently, LTBP2 levels were significantly higher in patients with a history of chronic kidney disease [0.029 (0.015–0.042) normalized level compared with 0.009 (0.005–0.014) normalized level; $P < 0.001$] and chronic heart failure (0.027 (0.013–0.039) normalized level compared with 0.010 (0.006–0.016) normalized level; $P < 0.001$]. No LTBP2 difference was observed between patients with known COPD and those without [0.011 (0.007–0.018) normalized level compared with 0.013 (0.007–0.027) normalized level; $P = 0.20$]. Weaker, albeit significant, correlations existed with systolic blood pressure ($r = -0.29$, $P < 0.001$), NYHA functional classes ($r = 0.18$, $P = 0.003$) and markers of infection and inflammation [neutrophile count: $r = 0.23$, $P < 0.001$; CRP (C-reactive protein): $r = 0.13$, $P = 0.04$]. No correlation existed with heart rate ($P = 0.60$), respiratory rate ($P = 0.27$), oxygen saturation ($P = 0.25$) and BMI (body mass index) at admission ($P = 0.77$). These correlations were independent of the

Table 1 Baseline characteristics divided into patients with and without AHF

Values are means ± S.D. or medians (IQR). ACEis/ARBs, angiotensin-converting enzyme inhibitors/angiotensin II type I receptor blockers.

Characteristic	Total ($n = 292$)	AHF ($n = 158$)	Non-AHF ($n = 134$)	P value
Age (years)	74 ± 12	78 ± 9	68 ± 13	<0.0001
Male sex	52%	51%	53	0.906
BMI (kg/m^2)	26.1 ± 6.2	26.6 ± 5.9	25.5 ± 6.5	0.124
Medical condition (n)				
Heart failure	71 (24%)	63 (40%)	8 (6%)	<0.0001
Coronary artery disease	82 (28%)	60 (38%)	22 (16%)	<0.0001
COPD	99 (34%)	43 (27%)	56 (42%)	0.006
Diabetes	53 (18%)	38 (24%)	15 (11%)	0.005
Hypertension	198 (68%)	123 (78%)	55 (56%)	<0.0001
Hyperlipidaemia	85 (29%)	52 (33%)	33 (25%)	0.165
Chronic kidney disease	82 (28%)	69 (44%)	15 (11%)	<0.0001
Initial clinical findings				
Heart rate (beats/min)	93 ± 23	93 ± 25	92 ± 19	0.495
Systolic pressure (mmHg)	138 ± 26	135 ± 27	140 ± 25	0.098
NYHA functional class (n)				
II	58 (20%)	15 (10%)	43 (32%)	<0.0001
III	118 (40%)	71 (45%)	47 (35%)	0.109
IV	116 (40%)	72 (45%)	43 (32%)	0.034
Oedema	124 (42%)	90 (57%)	34 (26%)	<0.0001
Rales	158 (54%)	101 (64%)	57 (43%)	<0.0001
Medication at admission (n)				
β-Blockers	113 (39%)	90 (57%)	23 (17%)	<0.0001
ACEis/ARBs	143 (49%)	98 (62%)	45 (34%)	<0.0001
Diuretics	153 (52%)	101 (64%)	52 (39%)	<0.0001
Laboratory findings				
Haemoglobin (g/l)	134 (119–145)	129 (113–143)	138 (125–151)	<0.0001
Neutrophile count (%)	80.31 (73.12–86.81)	80.27 (74.51–86.66)	80.35 (69.90–87.40)	0.387
C-reactive protein (μg/ml)	19.3 (6.2–76.7)	18.3 (6.2–55.7)	20.9 (6.2–101.9)	0.320
Creatinine (μmol/l)	84 (65–119)	99 (79–149)	70 (55–92)	<0.0001
Cystatin C*	1.00 (0.75–1.49)	1.23 (0.89–1.79)	0.82 (0.61–1.14)	<0.0001
eGFR (ml/min/1.73 m^2)	67 (44–89)	54 (36–73)	80 (63–112)	<0.0001
BNP (pmol/l)	349 (89–1121)	976 (467–1925)	81 (39–181)	<0.0001
NT-proBNP (pmol/l)	1656 (314–6105)	5757 (1924–13243)	300 (76–974)	<0.0001
LTBP2*	0.012 (0.006–0.024)	0.017 (0.010–0.033)	0.008 (0.004–0.014)	<0.0001

*Plasma levels were measured using SRM-based methodology and hence there are no units.

primary cause of dyspnoea and persisted in AHF and non-AHF patients.

LTBP2 levels and prognostic value of LTBP2 on short-term outcome

At 30 days, 29 patients (10%) had died: 20 AHF and nine non-AHF patients. Pulmonary deaths ($n = 9$), heart failure ($n = 7$), cancer ($n = 6$) and myocardial infarction ($n = 4$) were the most common causes of death. Non-survivors had significantly higher LTBP2 levels than survivors in the overall population ($P < 0.001$), the AHF subgroup ($P < 0.001$) and patients with dyspnoea of pulmonary origin ($P = 0.011$). As shown in Figure 2(A), LTBP2 levels were especially elevated in patients dying of pulmonary causes [0.011 [0.006–0.021] normalized level in survivors compared with 0.021 (0.012–0.028) normalized level for cardiac death and 0.066 (0.043–0.078) normalized level for pulmonary death]. In contrast, NP levels did not differ significantly between patients dying of cardiac or pulmonary causes [NT-proBNP, 11941 (3338–20973) pg/ml compared with 16195 (4897–25909) pg/ml; $P = 0.39$) (Figure 2B).

ROC curve analyses were performed to assess the potential of LTBP2 levels to predict all-cause short-term mortality. In Figure 3(A), the AUCs to predict 30-day

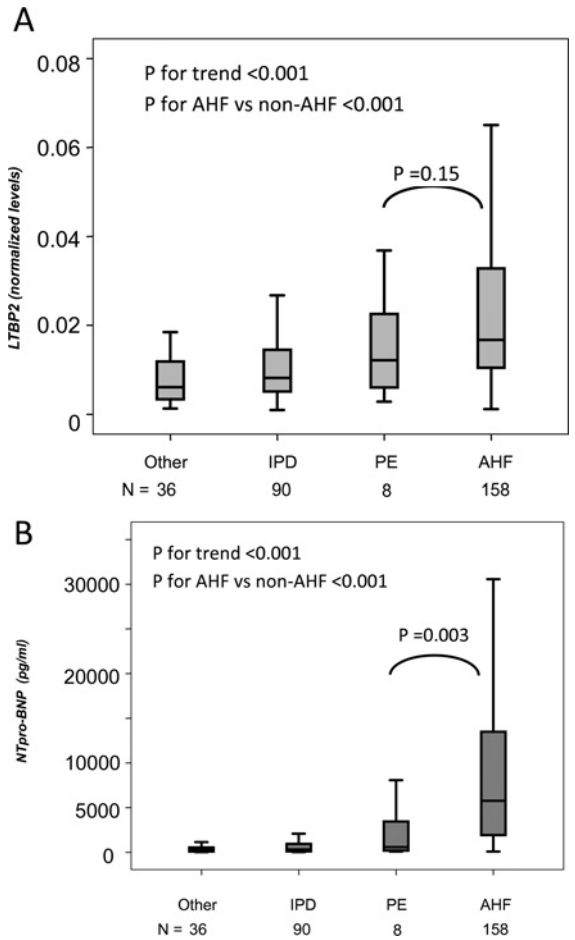

Figure 1 Box plots showing (A) LTBP2 and (B) NT-proBNP levels according to causes of dyspnoea

IPD, inflammatory pulmonary disease; PE, pulmonary embolism. The levels on the *y*-axis represent LTBP2 levels as measured by SRM-based methodology.

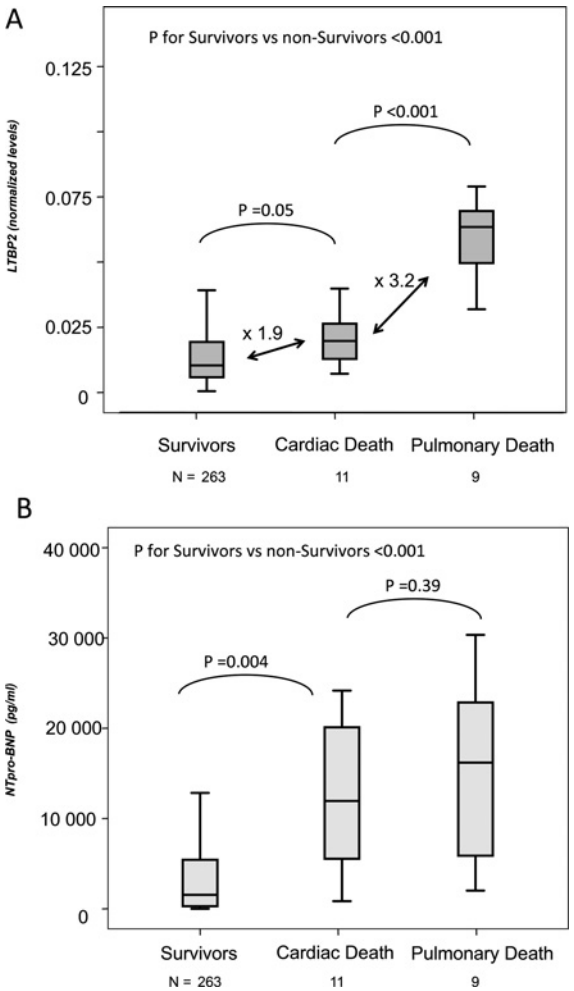

Figure 2 Box plots showing (A) LTBP2 and (B) NT-proBNP levels in 30-day survivors, cardiac non-survivors and pulmonary non-survivors

The LTBP2 levels on the *y*-axis represent LTBP2 levels as measured by SRM-based methodology.

all-cause mortality are illustrated for LTBP2 [0.79 (95 % CI, 0.70–0.87)], NT-proBNP [0.75 (95 % CI, 0.65–0.84)] and BNP [0.62 (95 % CI, 0.51–0.73)]. Figures 3(B) and 3(C) display the AUCs for the prediction of cause-specific mortality. ROC curve analyses demonstrated an AUC of 0.95 (95 % CI, 0.91–0.98) for LTBP2 to predict 30-day pulmonary mortality, which was significantly higher than the AUCs observed for NT-proBNP [0.84 (95 % CI, 0.75–0.94)] and BNP [0.63 (95 % CI, 0.48–0.77)] ($P = 0.04$ and <0.001 respectively).

LTBP2 levels and prognostic value of LTBP2 on 1-year outcome

Overall 80 (27 %) patients died during the first year of follow up; 58 AHF and 22 non-AHF patients. Heart failure ($n = 28$), myocardial infarction ($n = 14$), pulmonary death ($n = 14$) and cancer ($n = 14$) were the most common causes of death. LTBP2 levels in non-survivors were significantly higher compared with survivors for the overall patient population ($P < 0.001$), AHF patients ($P < 0.001$) and non-AHF ($P = 0.021$) patients. Again, there was a trend towards higher LTBP2 values in patients dying of pulmonary causes [0.009 (0.006–0.016) normalized level in survivors compared with 0.025 (0.016–0.037) normalized level for cardiac death and 0.052 (0.017–0.071) normalized level for pulmonary death] (Figure 4). NP levels did not separate between causes of death [NT-proBNP, 7785 (1920–22 584) pg/ml compared with 9757 (3772–18 609) pg/ml; $P = 0.52$]. Mortality according to LTBP2 level deciles is shown in Figure 5.

ROC curve analyses were performed to assess the potential of LTBP2 levels to predict all-cause and cause-specific 1-year mortality. Importantly, the prognostic potential of LTBP2 [AUC, 0.77 (95 % CI, 0.70–0.83)] was comparable with NT-proBNP [AUC, 0.77

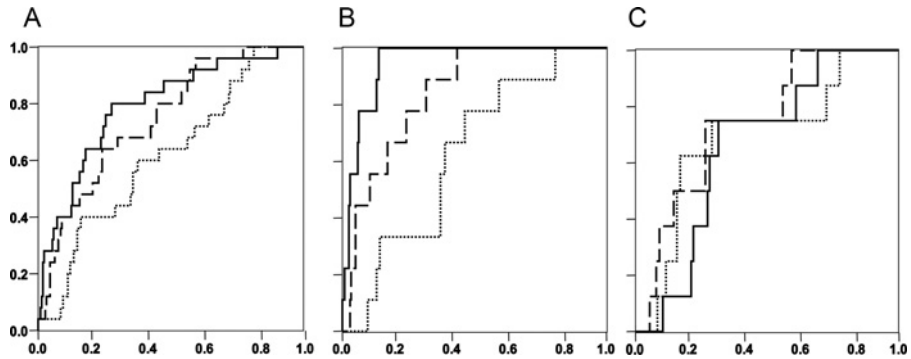

Figure 3 ROC curves displaying the potential of LTBP2 (continuous line) NT-proBNP (broken line) and BNP (dotted line) to predict 30-day all-cause mortality (A), pulmonary death (B) and cardiac death (C)

Sensitivity is shown on the *y*-axis and 1—specificity on the *x*-axis.

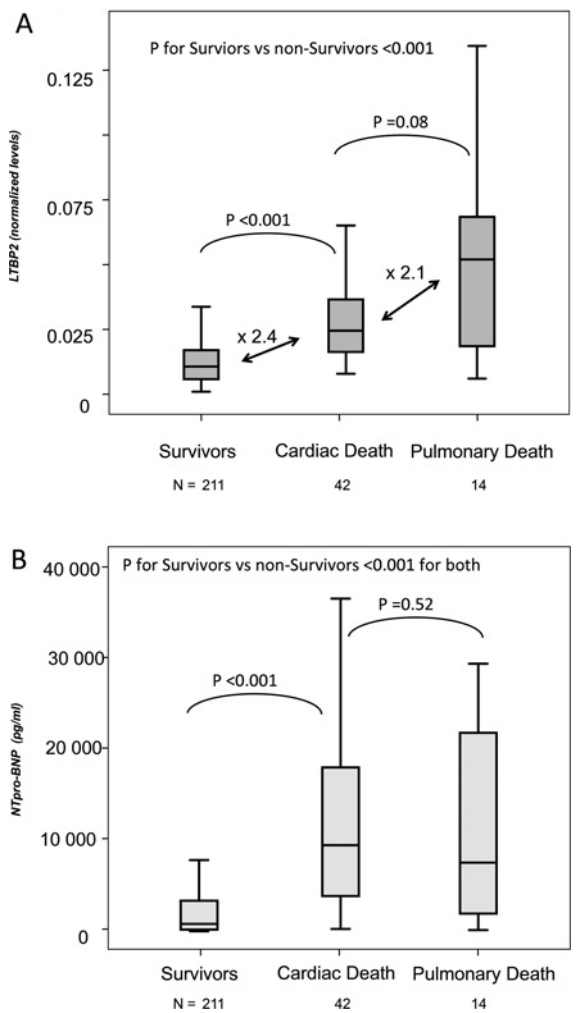

Figure 4 Box plots showing (A) LTBP2 and (B) NT-proBNP levels in 1-year survivors, cardiac non-survivors and pulmonary non-survivors

The LTBP2 levels on the *y*-axis represent LTBP2 levels as measured by SRM-based methodology.

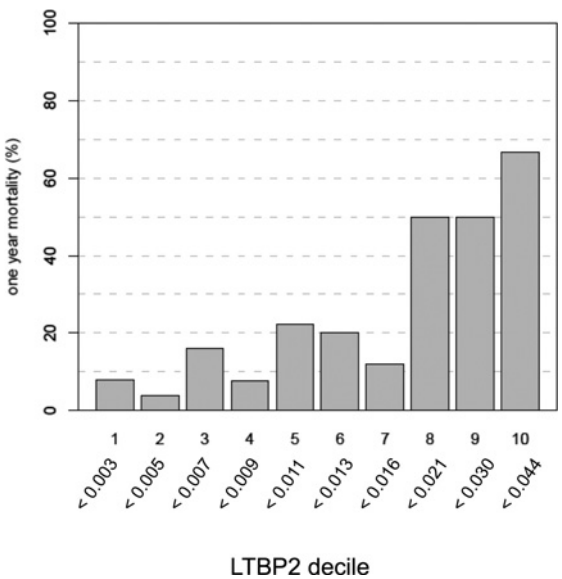

Figure 5 Relationship between LTBP2 deciles and 1-year all-cause mortality

(95 % CI, 0.71–0.84)] and BNP [AUC, 0.71 (95 % CI, 0.64–0.79)] for the prediction of all-cause (Figure 6A) and cardiac (Figure 6C) mortality (AUC, 0.77, 0.79, 0.80 and respectively) and tended to be superior for the prediction of pulmonary death (Figure 6B) (AUC, 0.80, 0.75 and 0.59 respectively; *P* value against NT-proBNP 0.37, *P* value against BNP 0.02). Importantly, the predictive potential of LTBP2 was independent of kidney dysfunction and persisted in patients with preserved kidney function [AUC, 0.77 (95 % CI, 0.70–0.83)].

Table 2 displays univariate and multivariable regression analyses for the prediction of 1-year all-cause mortality. In a multivariate Cox regression analysis, LTBP2 was the strongest independent predictor of death [HR (hazard ratio), 3.76 (95 % CI, 2.13–6.64); $P < 0.0001$] and exceeded the predictive potential of markers of kidney

Table 2 Univariate and multivariable regression analysis for the prediction of 1-year mortality

LVEF, left ventricular ejection fraction.

	Univariate		Multivariable	
Variable	HR (95 % CI)	*P* value	HR (95 % CI)	*P* value
Age (years)	2.49 (1.73–3.58)	<0.0001		
Weight at admission (kg)	0.73 (0.55–0.96)	0.0281		
Weight at discharge (kg)	0.52 (0.35–0.79)	0.0017		
BMI (kg/m^2)	0.66 (0.49–0.887)	0.0057	0.55 (0.4–0.768)	0.0004
Systolic blood pressure (mmHg)	0.60 (0.44–0.83)	0.0017		
Diastolic blood pressure (mmHg)	0.72 (0.54–0.98)	0.0336		
Oxygen saturation (%)	0.87 (0.75–1.01)	0.0629		
Oxygen therapy (yes/no)	1.51 (1.31–1.75)	<0.0001		
Respiratory rate (per min)	1.43 (1.16–0.76	0.0008		
Myoglobin	1.83 (1.44–2.32	<0.0001		
Potassium (mmol/l)	1.19 (1.12–1.26)	<0.0001	1.11 (1.03–1.18)	0.0037
eGFR (ml/min per 1.73 m^2)	0.57 (0.46–0.71)	<0.0001	1.72 (1.02–2.9)	0.0404
Blood urea nitrogen (mmol/l)	2.51 (1.91–3.29)	<0.0001	2.13 (1.19–3.84)	0.0112
Uric acid (μmol/l)	1.87 (1.36–2.57)	0.0001		
Albumin (g/l)	0.60 (0.49–0.73)	<0.0001		
Haemoglobin (g/l)	0.71 (0.59–0.84)	<0.0001		
Haematocrit (%)	0.66 (0.53–0.84)	0.0005		
LVEF (%)	0.64 (0.42–0.99)	0.0467		
Troponin T (μg/l)	1.81 (1.48–2.21)	<0.0001		
Cystatin C*	2.20 (1.65–2.92)	<0.0001		
LTBP2*	3.46 (2.47–4.85)	<0.0001	3.76 (2.13–6.64)	<0.0001
BNP (pg/ml)	2.97 (2.03–4.34)	<0.0001		
NT-proBNP (pg/ml)	4.20 (2.78–6.35)	<0.0001		
CRP (mg/l)	1.64 (1.18–2.26	0.0028		

*Plasma levels were measured using SRM-based methodology and hence there are no units.

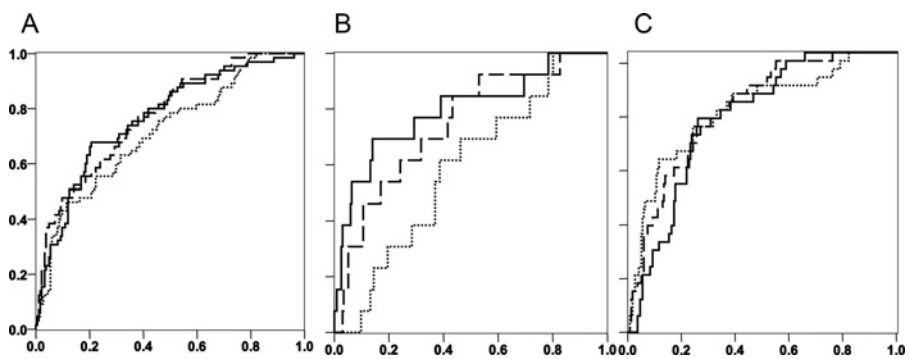

Figure 6 ROC curves displaying the potential of LTBP2 (continuous line) NT-proBNP (broken line) and BNP (dotted line) to predict 1-year all-cause mortlaity (A), pulmonary death (B) and cardiac death (C)

Sensitivity is shown on the *y*-axis and 1 − specificity on the *x*-axis.

function {eGFR (estimated GFR) HR, 1.72 ($P = 0.04$); urea HR 2.1 ($P = 0.01$)} and NT-proBNP. Tertiles of LTBP2 levels clearly separated all-cause (Figure 7A), pulmonary (Figure 7B) and cardiac (Figure 7C) decedents from survivors.

DISCUSSION

In the present study, we specifically examined the diagnostic and prognostic potential of LTBP2 in patients presenting with acute dyspnoea.

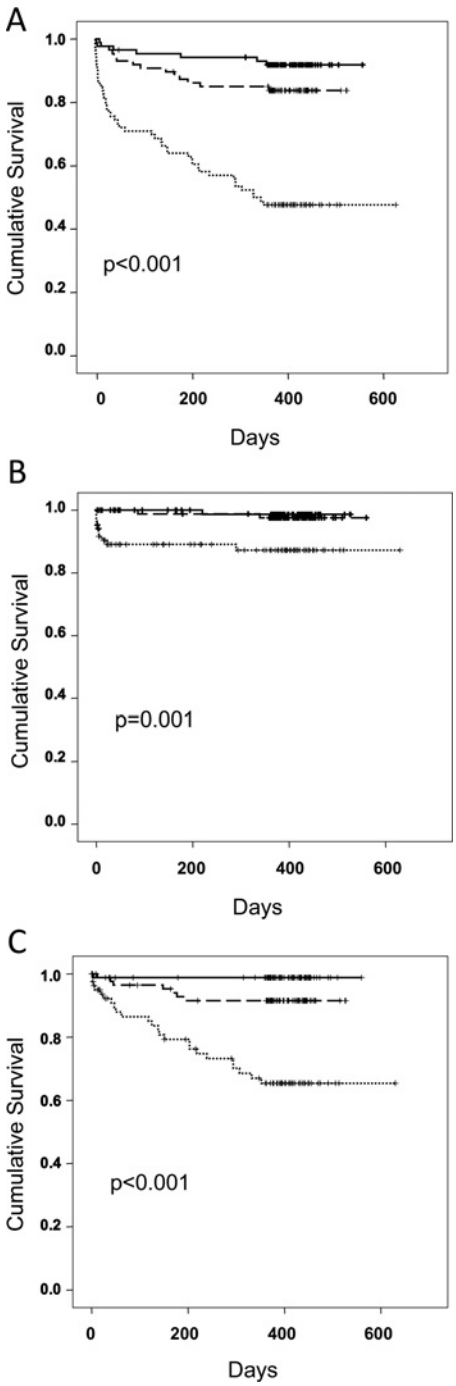

Figure 7 Kaplan–Meier survival curves displaying 1-year all-cause mortality (A), pulmonary death (B) and cardiac death (C) according to the LTBP2 tertiles

First tertile (continuous line), second tertile (broken line) and third tertile (dotted line) are shown.

There are several key findings in this study. First, LTBP2 levels increase steadily with increasing cardiac haemodynamic stress. Nevertheless, the accuracy of LTBP2 to diagnose AHF as the cause of acute dyspnoea is limited. Secondly, LTBP2 levels are significantly elevated in patients dying during short- and long-term follow-up. This increase is observed independent of the cause of dyspnoea. Thirdly, LTBP2 levels are especially elevated in patients dying of pulmonary causes. Fourthly, this leads to LTBP2 levels powerfully predicting short-term pulmonary death. Fifthly, during long-term follow-up LTBP2 levels remain strong and independent predictors of all-cause mortality.

LTBP2 was recently discovered in an unbiased proteomics search for novel heart failure-related markers [5]. It is part of the LTBP family which is emerging as a substantial and complex group of ECM proteins. Additionally, the LTBP family plays a pivotal role in controlling and directing the activity of TGFβ [6]. Importantly, however, LTBP2 is the only member of the LTBP family without TGFβ-binding capabilities [12]. In addition, the phenotype of LTBP2 gene-targeted mice is marked by early embryonic lethality and differs significantly from the phenotype displayed by TGFβ-knockout mice [7]. Hence LTBP2 appears to possess important TGFβ-independent tasks. During *in situ* hybridization experiments, LTBP2 commonly co-localizes with tropoelastin within the skin, heart, lung and large arterial vessels [7] suggesting a role in ECM structure and cell adhesion. Interestingly, the highest levels of LTBP2 are consistently found in lung tissue [7], suggesting the lung as an important source of circulatory LTBP2 levels. The MS assay used for LTBP2 detection in the present study specifically detects the N-terminal part of the protein, which is highly resistant to proteolysis and acts as the fragment that adheres to the ECM [13]. The N-terminal part of the protein is generally surrounded by ECM. Circulating LTBP2 levels might therefore represent a spill-over of excessive or damaged ECM LTBP2 from the lung.

This pulmonary origin of LTBP2 could explain the LTBP2 elevation in patients at high-risk of short-term pulmonary death. It should be noted that various pathophysiological pathways appear to be able to induce LTBP2-releasing acute pulmonary injury. In fact, in the setting of AHF, increased hydrostatic pressure has recently been shown to be able to induce ultrastructural changes of the blood–gas barrier [14] and consequently lead to pulmonary parenchymal inflammation [15]. In this cohort, two-thirds of the patients dying of pulmonary causes during the first 30 days of follow-up suffered from AHF, whereas one-third were diagnosed with pneumonia or acute exacerbation of COPD as the cause of dyspnoea. This distribution closely mirrors the distribution of final diagnoses in the overall cohort (AHF 54 %, acute exacerbation of COPD and pneumonia 21 %).

A marker of acute lung injury and pulmonary risk in patients with acute dyspnoea would be of great clinical importance and complementary to NPs, which quantify haemodynamic cardiac stress [16]. Similarly, the prognostic potential of NPs is predominantly based on

identifying patients at risk of cardiovascular events [17–19]. Consequently, we found NP levels to inadequately assess the risk of short-term pulmonary death. The addition of LTBP2 levels could provide the clinician with an early warning sign for pulmonary risk highlighting the importance of fast and adequate volume depletion in AHF patients, targeted antimicrobial therapy in pneumonia patients and further diagnostics and close observation in patients with multifactorial dyspnoea. Additionally, AHF patients at high pulmonary risk might benefit from pre-emptive antibiotic therapy and anti-inflammatory drugs to halt the progression from hydrostatic-injury-induced parenchymal inflammation to lethal pulmonary infection.

During long-term follow-up, the association between LTBP2 levels and pulmonary mortality weakened, but LTBP2 remained a strong and independent predictor of all-cause mortality. In fact, the separation of the pulmonary Kaplan–Meier survival curves is solely based on the short-term pulmonary excess mortality of patients in the highest LTBP2 tertile. A possible explanation for this observation lies in the reversibility of the acute ultrastructural changes of the blood–gas barrier in hydrostatic pulmonary injury [20]. This observation suggests that pulmonary death in patients with AHF might be linked to leaks in the blood–gas barrier and that the occurrence of pulmonary deaths decreases after the repair of the acute injury.

Additionally, extrapulmonary release of circulating LTBP2 levels might contribute to the weaker long-term association with pulmonary death. In fact, heart and kidneys also produce LTBP2, and LTBP2 transcript levels have been shown to rise early in the chronic kidney and heart failure disease continuums [7]. In the heart, LTBP2 levels are increased in hypertrophied left ventricles, an early step in the development of heart failure. Similarly LTBP2 expression in the kidney is up-regulated in correlation with the disease severity in animal models of chronic kidney disease [21]. These extrapulmonary production sites may help to explain the strong association of circulating LTBP2 levels with cardiac stress and markers of renal function observed in this study. Importantly, cardiac stress and renal impairment have repeatedly been shown to carry prognostic importance in various disease states [22–24]. Hence, while pulmonary LTBP2 release appears to underlie the strong short-term pulmonary-risk stratification, cardiac and renal LTBP2 release seems to contribute to the long-term predictive potential of this promising new marker. By integrating acute pulmonary injury, cardiac stress and renal impairment, circulating LTBP2 levels provide strong and independent long-term risk assessment.

Several limitations merit consideration. First, this was an observational single-centre study. However, since baseline characteristics and mortality rates are similar to other published acute dyspnoea cohorts, we consider our patient population representative [2,4,25]. Nevertheless our results represent the first description of LTBP2 in patients with acute dyspnoea and will need to be validated in larger, multicentre studies. Secondly, currently only semi-quantitative measurements of LTBP2 by MS are available. Immunoassays are in development and should soon simplify research and subsequently allow routine clinical use. Thirdly, treating physicians were blinded to the LTBP2 test results. We can therefore not assess the impact of a biomarker guided treatment strategy on therapy changes, treatment costs and long-term outcome.

In conclusion, the plasma levels of LTBP2 present a novel and powerful predictor of all-cause mortality, and particularly pulmonary death. Cause-specific prediction of death with this potential marker of acute lung injury would enable targeted prevention, for example with pre-emptive antibiotic therapy.

AUTHOR CONTRIBUTION

Tobias Breidthardt, Griet Vanpoucke, Koen Kas and Christian Mueller participated in study concept and design the acquisition of data, analysis and interpretation of data, drafting of the paper, critical revision of the paper for important intellectual content, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Gregoire Thomas, Piet Moerman, Mihael Potocki, Tamina Mosimann, Ronny Ziller, Thenral Socrates and Beatrice Drexler participated in the acquisition of data, analysis and interpretation of data and critical revision of the paper for important intellectual content. Wouter Laroy conducted the LTBP2 measurements. Alexandre Mebazaa participated in analysis, interpretation of data, drafting of the paper and critical revision of the manuscript for important intellectual content. All authors read and approved the final paper.

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