Plasma haptoglobin level can augment NT-proBNP to predict poor outcome in patients with severe acute decompensated heart failure

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ABSTRACT

To evaluate the use of plasma haptoglobin (Hp) levels and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting survival in patients with severe acute decompensated heart failure (AHF). Management of AHF is challenging. Identifying markers associated with patient prognosis in this disease is clinically important. In this prospective observational study, plasma Hp and NT-proBNP levels were measured. Receiver operating characteristic (ROC) curves were used to identify cut-offs of Hp and NT-proBNP with the greatest specificity and sensitivity for predicting overall survival and cardiovascular-related survival. The cut-off values were tested in patients with AHF (n=41). The cut-off value with the greatest specificity and sensitivity with respect to overall survival and for cardiovascular-related survival for Hp was 177.1 ng/mL for both outcomes and for NT-proBNP was 34 246.0 pg/mL and 11 848.5 ng/mL, respectively. Using these cut-off values, this study found that patients with lower baseline Hp levels (<177.1 ng/mL) or higher baseline NT-proBNP (≥34 246 pg/mL) were more likely to have shorter overall survival. Similarly, patients with <177.1 ng/mL of Hp and ≥11 848.5 pg/mL of NT-proBNP had the highest risk of death related to cardiovascular disease. Our findings indicate that Hp and NT-proBNP using specific cut-off values for AHF can be used to determine risk of survival in these patients.

Significance of this study

What is already known about this subject?

- Acute decompensated heart failure (AHF) still remains a major clinical challenge, especially with regard to treatment, prognosis and risk stratification.
- Clinical assessment of AHF is notoriously difficult, and even after the diagnosis is established, it is difficult to predict which patients will die or suffer further cardiovascular events.
- Detecting serum N-terminal pro- B-type natriuretic peptide (NT-proBNP) levels is widely used in the early diagnosis, prognosis and risk stratification of patients with AHF.

What are the new findings?

- The cut-off value with the greatest specificity and sensitivity with respect to overall survival and for cardiovascular-related survival for plasma haptoglobin (Hp) was 177.1 ng/mL for for both outcomes and for NT-proBNP was 34 246.0 pg/mL and 11 848.5 ng/mL, respectively.
- Patients with lower baseline Hp levels (<177.1 ng/mL) and higher baseline NT-proBNP (11 848.5 ng/mL) were more likely to have lower overall survival.
- For cardiovascular-related survival, better survival time was also seen in patients with Hp levels≥177.1 ng/mL than patients with lower Hp levels, and longer survival time was observed in patients with NT-proBNP levels<11 848.5 pg/mL than patients with higher NT-proBNP levels.

How might these results change the focus of research or clinical practice?

- Hp and NT-proBNP levels can be used to determine risk of survival of patients with AHF.

INTRODUCTION

Approximately 1%–2% of the adult population in high-income countries has heart failure (HF), and this prevalence rises to >10% among the elderly (ie, persons aged >70 years of age).[1 2] Hospitalizations for HF have also risen steadily, with >1 million patients in the USA reported to have HF in 2004.[3 4] With greater knowledge with respect to the causes of congestive HF, improved treatment options have been developed that extend the life of the patient. However, acute decompensated heart failure (AHF) still remains a major clinical challenge, especially with regard to treatment, prognosis and risk stratification.

AHF is a syndrome characterized by a gradual or rapid change in HF symptoms resulting from severe pulmonary congestion due to elevated left ventricular (LV) filling pressures. Although there is no current consensus regarding its epidemiology or its pathophysiology, AHF is often triggered by identifiable factors, such as non-compliance

with medication, diet, aggravated hypertension, acute coronary syndromes, arrhythmias and systemic inflammation.[3–7] It can occur in patients with either preserved or reduced LV systolic function. The primary treatment goals for AHF are aimed at reducing pulmonary capillary wedge pressure and increasing cardiac output.[4]

Clinical assessment of AHF is notoriously difficult, and even after the diagnosis is established, it is difficult to predict which patients will die or suffer further cardiovascular events. Factors found associated with mortality include increasing age, a history of diabetes mellitus or renal dysfunction, higher functional disability measures such as New York Heart Association (NYHA) class, lower LV ejection fraction (LVEF), lower sodium concentrations, lower body mass index and lower blood pressure.[8–10] However, none of these factors is a strong predictor of mortality and consequently, it is of clinical importance to identify other prognostic markers.

Two factors that have garnered attention as important prognostic predictors of mortality and cardiovascular events for all stages of AHF are B-type natriuretic peptide (BNP) and its precursor form, N-terminal pro-brain natriuretic peptide (NT-proBNP).[11 12] BNP is a neurohormone secreted primarily by the ventricles in response to myocardial tension and increased intravascular volume.[10 13–16] Increasing BNP and NT-proBNP concentrations are linearly associated with poor prognosis in patients with AHF.[13 17] In fact, detecting serum NT-proBNP levels is widely used in the early diagnosis, prognosis and risk stratification of patients with AHF.[18]

In addition to fluid overload, systemic low-grade inflammation is also a common feature in patients with chronic AHF.[19] Haptoglobin (Hp), an alpha-2 glycoprotein, is the major hemoglobin (Hb) binding protein and is one of the acute phase proteins that increase during inflammation.[20] Its primary function is to bind to extracorpuscular free Hb, thereby attenuating its oxidative and inflammatory effects.[21] The Hb-Hp complex is rapidly removed by CD163 scavenger receptors on monocyte and macrophages.[22] Hp also exerts an antioxidant effect via direct inhibition of low-density lipoprotein (LDL) oxidation and prostaglandin synthesis.[15 16 23–25] Holme *et al*[26] found that serum Hp had as much prognostic predictive value in patients with acute myocardial infarction (AMI) and stroke as total serum cholesterol. They also suggested that serum Hp was also a risk factor for HF.[26]

Recent studies have found that plasma levels of Hp are elevated in patients with both abdominal aortic aneurysm[27] and coronary artery disease.[28] However, the role of Hp levels in predicting clinical outcomes in subjects with AHF is still unclear. The aim of this study was to investigate whether plasma levels of Hp in combination with NT-proBNP can be used as prognostic markers for survival in patients with AHF.

MATERIALS AND METHODS

Study participants

This study was performed in accordance with the Declaration of Helsinki. All patients who were enrolled in the study gave their written informed consent prior to study participation.

Between February 2011 and November 2013, all patients who were admitted to the Coronary Care Unit (CCU) of our hospital with a primary diagnosis of NYHA functional classification III or IV AHF were screened. Patients who had concomitant acute coronary syndrome, a documented myocardial infarction event within 3 months of study start, hematological disorders or severe sepsis were excluded.

The end point of the study was death either due to cardiovascular causes (ie, fatal myocardial infarction, cerebral vascular accident, refractory HF and pulseless ventricular tachycardia or fibrillation) or other causes, that is, refractory respiratory failure, uremia, malignancy and infection with septic shock.

Echocardiography

Echocardiograms were performed by trained sonographers using the IE33 ultrasound system (Philips Healthcare, 5680 DA Best, The Netherlands). Each patient was studied in the left lateral position to obtain optimal visualization of the left ventricle. LV end-diastolic and end-systolic volumes were acquired using Simpson's method in the apical four-chamber view per the American Society of Echocardiography's recommendations.[29] Ejection fraction was calculated using the following: (end-diastolic volume–end-systolic volume)/end-diastolic volume×100%.

Clinical chemistry

Overnight fasting blood samples were obtained from all patients on admission, and at 1 month and 3 months follow-up after discharge and mixed with 0.1% ethylenediaminetetraacetic acid for measurement of total cholesterol, high-density lipoprotein, LDL and triglyceride concentrations using standard techniques. Plasma C reactive protein (CRP) concentrations were determined by IMMAGE high-sensitivity CRP assay (Beckman Coulter, Fullerton, California, USA). Plasma NT-proBNP levels were determined by electrochemiluminescence immunoassay. Estimated glomerular filtration rate (eGFR) was calculated using a modified version of the modification of diet in renal disease equation:

$$eGFR = 186 \times PCR\text{-}1.154 \times age - 0.203 \times (0.742 \text{ if female}).$$[30]

Haptoglobin phenotyping

Hp phenotyping was performed, as previously described, using native polyacrylamide gel electrophoresis (PAGE) with Hb-supplemented plasma.[31] Briefly, overnight fasting blood samples were obtained from all patients before catheterization and mixed with 0.1% ethylenediaminetetraacetic acid. Plasma was isolated immediately by centrifuge and then stored at −20°C. Subsequently, 7 µL of plasma was premixed with 5 µL of 8 mg/mL Hb and equilibrated with 3 µL of sample buffer (0.625 mol/L Tris-base, pH 6.8, 50% glycerol (v/v) and 0.125 mg/L bromophenol blue). The mixture was run on a 7% native polyacrylamide gel (pH 8.8), with 5.5% polyacrylamide (26.5:1; acrylamide: bisacrylamide) used as a top stacking gel (pH 6.8).

Electrophoresis was performed at an initial voltage of 120 V, which was increased up to 150 V when the dye

Table 1 Study cohort baseline characteristics

Study characteristics	Survivors (n=31)	Non-survivors (n=10)	P values
Age, years	82.0 (68.0, 85.0)	84.5 (79.0, 88.0)	0.134
Male gender, n (%)	22 (71.0%)	6 (60.0%)	0.460
Body mass index, kg/m^2	25.0 (23.1, 27.0)	26.7 (20.7, 30.1)	0.688
NYHA			
III	15 (48.4%)	2 (20.0%)	Ref.
IV	16 (51.6%)	8 (80.0%)	0.162
Hp phenotype			
1–1	5 (16.1%)	1 (10.0%)	Ref.
2–1	12 (38.7%)	3 (30.0%)	0.815
2–2	14 (45.2%)	6 (60.0%)	0.515
Smoking, n (%)	7 (22.6%)	0 (0.0%)	NA
Comorbidity			
Hypertension, n (%)	24 (77.4%)	9 (90.0%)	0.430
Diabetes, n (%)	21 (67.7%)	6 (60.0%)	0.674
CVA, n (%)	1 (3.2%)	2 (20.0%)	0.076
CAD, n (%)	14 (45.2%)	1 (10.0%)	0.090
Pneumonia, n (%)	6 (19.4%)	0 (0.0%)	NA
UTI, n (%)	2 (6.5%)	2 (20.0%)	0.106
Drug use			
β-Blocker, n (%)	17 (54.8%)	3 (30.0%)	0.204
Statin, n (%)	8 (25.8%)	1 (10.0%)	0.343
RAS blockade, n (%)	16 (51.6%)	3 (30.0%)	0.284
Inotropics, n (%)	11 (35.5%)	5 (50.0%)	0.425
Blood test			
Hemoglobin, g/dL	10.2 (8.8, 13.3)	9.2 (8.4, 12.8)	0.798
CRP, mg/dL	1.5 (0.3, 4.7)	1.2 (0.4, 2.0)	0.250
Cr, mg/dL	2.0 (1.3, 3.2)	2.2 (1.9, 2.7)	0.812
eGFR, ml/min	26.6 (18.1, 35.0)	24.6 (14.3, 32.1)	0.348
Cholesterol, mg/dL	136.5 (114.0, 166.0)	156.0 (118.0, 166.0)	0.523
LVEF, %	35.0 (28.0, 49.0)	45.5 (40.0, 53.0)	0.197
Haptoglobin (ng/mL)	188.5 (133.9, 214.4)	124.3 (98.1, 167.4)	0.048*
NT-proBNP (pg/mL)	9822.0 (4932.0, 17 467.0)	20 124.5 (7984.0, 40 083.0)	0.082

Categorical data are expressed as number and percentage, continuous data are expressed as median and IQR. P value was presented by Cox proportional hazards regression model.

*Indicates a significant factor.

CAD, coronary artery disease, Cr, creatinine; CRP, C reactive protein; CVA, cerebrovascular accident; eGFR, estimated glomerular filtration rate; Hp, haptoglobin; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RAS, renin-angiotensin system; UTI, urinary tract infection.

front reached the separating gel. After electrophoresis, the Hp-Hb complex was visualized by shaking the gel in freshly prepared peroxidase substrate (0.05% 3,3′-diaminobenzidine (w/v) and 0.07% hydrogen peroxide (v/v) in phosphate buffered saline). The results were confirmed by western blot analysis using an α-chain-specific monoclonal antibody prepared by ourselves.[31 32]

Haptoglobin purification

Hp was purified from plasma by chromatography on a monoclonal antibody-based affinity column followed by high-performance liquid chromatography (HPLC) as described previously.[31] Briefly, 2 mL of filtered human plasma was loaded onto the antibody affinity column (10 mL bed volume) at room temperature. The column was then washed with 50 mL of 20 mmol/L phosphate buffer containing 0.2 mol/L NaCl (pH 7.4), and then eluted with 50 mL of freshly prepared 0.15 mol/L NaCl solution (pH 11). Fractions (5 mL) were collected in tubes containing 0.25 mL of 1 mol/L Tris–HCl buffer (pH 6.8) to immediately neutralize the base. Pooled fractions containing Hp were then concentrated to a final volume of 1 mL using Centricon tubes (Millipore, Cork, Ireland) and filtered through a 0.45 µm membrane. Gel-filtration HPLC with a Superose-12 column (1×30 cm) (Pharmacia, Uppsala, Sweden) was then performed. The homogeneity of each isolated Hp type was >95% as judged by sodium dodecyl sulfate-PAGE.

Measurements of human plasma Hp concentration

Plasma free Hp concentrations were measured using a phenotype-matched standard sandwich ELISA, as previously described.[31] The ELISA detected total Hp, including both Hb-conjugated Hp and free Hp. Due to the diverse immunochemical structures among Hp types, phenotyping was performed in all the patients and a type-matched Hp

Table 2 Results of receiver operating characteristic curve

Months	Number of event < cut-off value/ total event	Optimal cut-off value	Sensitivity % (95% CI)	Specificity % (95% CI)	Area under curve % (95 % CI)	P values
Haptoglobin						
Overall survival						
1	2/3	116.5 ng/mL	66.7 (0.0 to 100.0)	84.2% (71.1 to 94.7)	71.9% (45.4 to 98.5)	0.211
3	4/6	116.5 ng/mL	66.7 (33.3 to 100.0)	88.6% (77.1 to 97.1)	77.1% (58.3 to 96.0)	0.036*
6	5/7	116.5 ng/mL	71.4 (28.6 to 100.0)	91.2% (79.4to 100.0)	80.3% (63.4 to 97.1)	0.013*
12	9/9	177.1 ng/mL	100.0 (100.0 to 100.0)	56.2% (37.5 to 71.9)	76.7% (61.2 to 92.3)	0.015*
18	10/10	177.1 ng/mL	100.0 (100.0 to 100.0)	58.1% (41.9 to 74.2)	77.1% (62.4 to 91.8)	0.011*
24	10/10	177.1 ng/mL	100.0 (100.0 to 100.0)	58.1% (41.9 to 74.2)	77.1% (62.4 to 91.8)	0.011*
Cardiovascular-related survival						
1	2/3	116.5 ng/mL	66.7% (0.0 to 100.0)	84.2% (71.1 to 94.7)	71.9% (45.4 to 98.5)	0.211
3	4/6	116.5 ng/mL	66.7% (33.3 to 100.0)	88.6% (77.1 to 97.1)	77.1% (58.3 to 96.0)	0.036*
6	4/6	116.5 ng/mL	66.7% (33.3 to 100.0)	88.6% (77.1 to 97.1)	77.1% (58.3 to 96.0)	0.036*
12	4/6	116.5 ng/mL	66.7% (33.3 to 100.0)	88.6% (77.1 to 97.1)	77.1% (58.3 to 96.0)	0.036*
18	7/7	177.1 ng/mL	100.0% (100.0 to 100.0)	52.9% (35.3 to 70.6)	76.9% (60.3 to 93.5)	0.027*
24	7/7	177.1 ng/mL	100.0% (100.0 to 100.0)	52.9% (35.3 to 70.6)	76.9% (60.3 to 93.5)	0.027*
N-terminal pro-B-type natriuretic peptide						
Overall survival						
1	0/3	11 848.5 pg/mL	100.0 (100.0% to 100.0)	57.9 (42.1 to 73.7)	74.6 (49.3 to 99.9)	0.161
3	2/6	11 848.5 pg/mL	66.7 (33.3% to 100.0)	57.1 (42.8 to 74.3)	57.1 (30.1 to 84.2)	0.580
6	2/7	11 848.5 pg/mL	71.4 (42.9% to 100.0)	58.8 (44.1 to 76.5)	63.9 (38.3 to 89.5)	0.253
12	4/9	16 933.5 pg/mL	55.6 (22.2% to 88.9)	71.9 (56.3 to 87.5)	62.5 (38.8 to 86.2)	0.257
18	6/10	34 246.0 pg/mL	40.0 (10.0% to 70.0)	96.8 (90.3 to 100.0)	67.1 (44.7 to 89.5)	0.108
24	6/10	34 246.0 pg/mL	40.0 (10.0 to 70.0)	96.8 (90.3 to 100.0)	67.1 (44.7 to 89.5)	0.108
Cardiovascular-related survival						
1	0/3	11 848.5 pg/mL	100.0 (100.0 to 100.0)	57.9% (42.1 to 73.7)	74.6 (49.3 to 99.9)	0.161
3	2/6	11 848.5 pg/mL	66.7 (33.3 to 100.0)	57.1% (42.8 to 74.3)	57.1 (30.1 to 84.2)	0.580
6	2/6	11 848.5 pg/mL	66.7 (33.3 to 100.0)	57.1% (40.0 to 74.3)	57.1 (30.1 to 84.2)	0.580
12	2/6	11 848.5 pg/mL	66.7 (33.3 to 100.0)	57.1% (42.8 to 74.3)	57.1 (30.1 to 84.2)	0.580
18	2/7	11 848.5 pg/mL	71.4 (28.6 to 100.0)	58.8% (41.2 to 76.5)	63.4 (38.1 to 88.8)	0.268
24	2/7	11 848.5 pg/mL	71.4 (42.9 to 100.0)	58.8% (41.2 to 73.5)	63.4 (38.1 to 88.8)	0.268

calibrator was used in clinical Hp determination as previously described.[31]

Statistical analysis

Continuous variables were presented as median and IQR, categorical variables were presented as counts and percentages. Univariate Cox proportional hazards regression model was used to investigate event risk, including age, gender, body mass index (BMI), NYHA functional class, Hp phenotype, smoking, comorbidities, drug uses and blood test. In Cox proportional hazards regression analysis, any cause of deaths was treated as events and survivors were treated as censored events. NT-proBNP and risk factor with statistical significance in univariate Cox proportional hazards regression model were further included in receiver operating characteristic (ROC) analyses by the timepoint of 1, 3, 6, 12, 18 and 24 months. All subjects were included, and assumed that subjects without events which had shorter follow-up time were survived to the point in time. The ROC curves were performed to detect the diagnostic performance using Hp and NT-proBNP; and the optimal cut-off values for distinguishing survival were selected by Youden index and used to define sensitivity and specificity. Hp and NT-proBNP were grouped by the optimal cut-off values and the Kaplan-Meier curves were used to evaluate the overall and cardiovascular-related survival between Hp and NT-proBNP groups. Statistical analysis was considered significant as the two-sided p value was <0.05. The statistical analyses were performed by IBM SPSS statistics V.22.0 software (IBM, Armonk, New York, USA) and pROC package in R language.

RESULTS

Study cohort characteristics of survivors versus non-survivors

Table 1 summarizes the baseline characteristics of the study cohort. Of the 41 patients recruited, 10 patients (6 males and 4 females) died during the follow-up period (median follow-up, 22 months) as the non-survivors group, including 7 patients' deaths were due to cardiovascular causes. The results of univariate Cox proportional hazards regression showed that age, gender, BMI, NYHA functional class, Hp phenotype, smoking, comorbidities and drug uses had no significantly OS benefit over their reference group (all p values $\geqq 0.076$). The median CRP levels were also

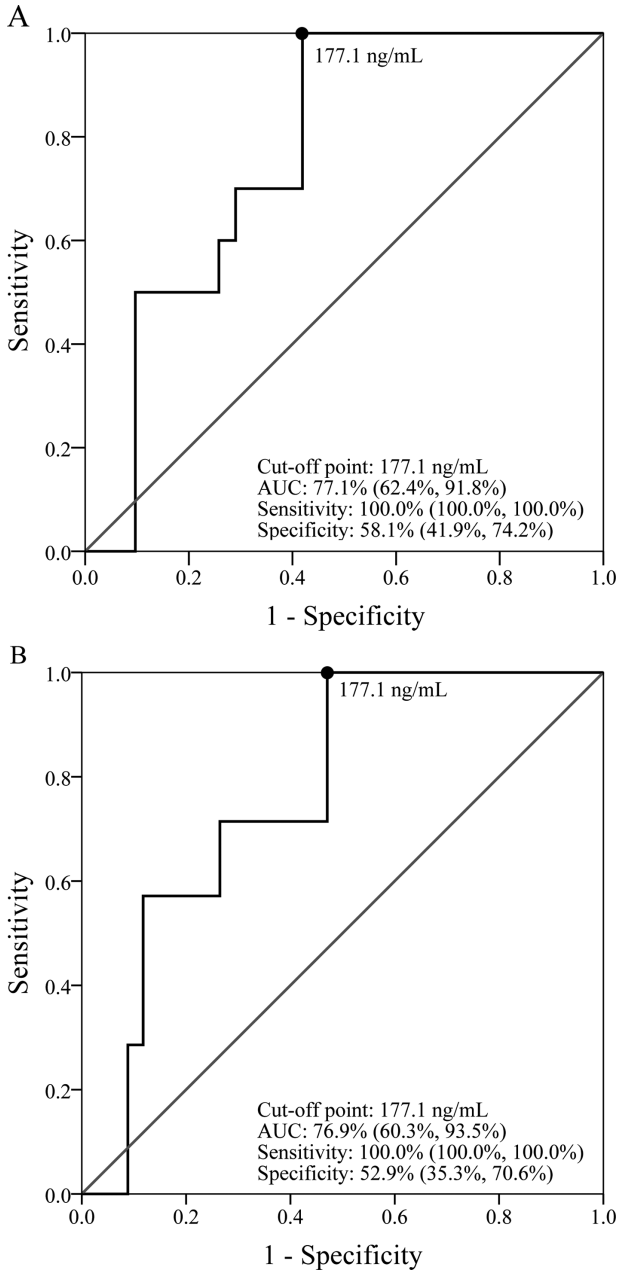

Figure 1 Receiver operating characteristic (ROC) curve for the optimal haptoglobin level cut-off (grouped in ≥177.1 ng/mL and <177.1 ng/mL) for identifying the influence of haptoglobin level on (A) overall survival and (B) cardiovascular-related survival at follow-up time point of 18 months. AUC, area under curve.

similar between the two groups (1.5 vs 1.2 mg/dL, respectively, p=0.250) as were NT-proBNP levels (p=0.082). The median Hp levels was significantly higher in survivors compared with non-survivors (188.5 vs 124.3 ng/mL, respectively, p=0.048).

Results of ROC curve of haptoglobin and NT-proBNP and survival

We used ROC analysis to identify cut-off values for both markers that maximize sensitivity and specificity for predicting overall survival and cardiovascular-related survival by different time points. The results of ROC curves for Hp and NT-proBNP are summarized in table 2 and figure 1. The cut-off value of 177.1 ng/mL for Hp overall survival and cardiovascular-related survival in time-point of 18 months, respectively were used. The area under curve (AUC) showed discriminatory power to distinguish survivors from non-survivors with Hp (overall survival: AUC=77.1% (62.4%–91.8%), p=0.011; cardiovascular-related survival: AUC=76.9% (60.3%–93.5%), p=0.027), and number of non-survivors in the Hp levels<177.1 ng/mL was higher than that in the Hp levels≥177.1 ng/mL (overall survival: 10 vs 0; cardiovascular-related survival: 7 vs 0). Using cut-off values for overall survival and cardiovascular-related survival of 34 246.0 and 11 848.5 pg/mL for NT-proBNP in time-point of 18 months. The AUC for overall survival was 67.1% (95% CI 44.7% to 89.5%, p=0.108) and for cardiovascular-related survival was 63.4% (95% CI 38.1% to 88.8%, p=0.268), and number of non-survivors in the NT-proBNP levels<34 246.0 ng/mL was higher than that in the NT-proBNP levels≥34 246.0 ng/mL (overall survival: 6 vs 4); and number of non-survivors in the NT-proBNP levels<11 848.5 ng/mL was lower than that in the NT-proBNP levels≥11 848.5 ng/mL (cardiovascular-related survival: 2 vs 5).

For Hp, the sensitivity and specificity for overall survival were 100.0% and 58.1% at the time point of 18 months, respectively, and for cardiovascular-related survival were 100.0% and 52.9%. For NT-proBNP, the sensitivity for cardiovascular-related survival was higher than that for overall survival (71.4% vs 40.0%, respectively), but the specificity of cardiovascular-related survival was lower than that for overall survival (58.8% vs 96.8%) at the time point of 18 months.

Kaplan-Meier analysis of Hp and NT-proBNP markers and survival time

Better overall survival time was observed in patients with Hp levels≥177.09 ng/mL than patients with lower Hp levels (figure 2A). For NT-proBNP, longer overall survival time was seen in patients with levels<34 246 pg/mL than in patients with higher NT-proBNP levels (figure 2B). For cardiovascular-related survival, better survival time was also seen in patients with Hp levels≥177.09 ng/mL than patients with lower Hp levels (figure 2C), and longer survival time was observed in patients with NT-proBNP levels<11 848.5 pg/mL than patients with higher NT-proBNP levels (figure 2D).

DISCUSSION

We used ROC analysis to define cut-off values for Hp and NT-proBNP that maximize the sensitivity and specificity of the two markers in predicting survival. The cut-off value with the greatest specificity and sensitivity for Hp with respect to overall survival and for cardiovascular-related survival was 177.1 ng/mL for both outcomes and for NT-proBNP was 34 246 pg/mL and 11 848.5 ng/mL, respectively. Using these cut-off values, this study showed that patients with lower Hp levels on admission (<177.1 ng/mL) and higher NT-proBNP (≥34 246 pg/mL) had reduced overall survival. Similarly, patients with <177.1 ng/mL of Hp and ≥11 848.5 pg/mL of NT-proBNP had the highest risk of death related to cardiovascular disease. Our findings

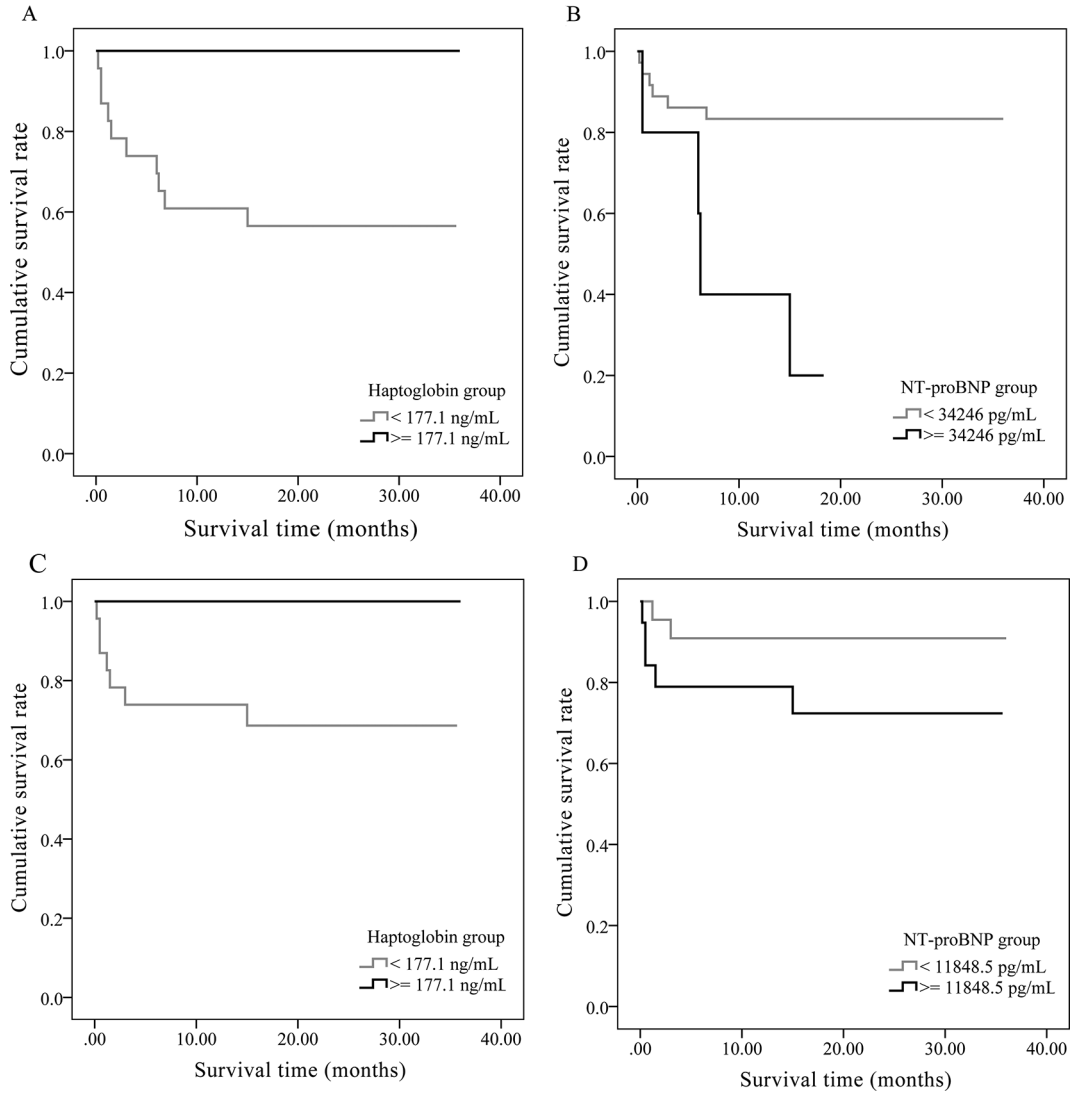

Figure 2 Kaplan-Meier curves for overall survival (A) by haptoglobin (Hp) level on admission (grouped in ≥177.1 ng/mL and <177.1 ng/mL), and (B) by N-terminal pro- B-type natriuretic peptide (NT-proBNP) level on admission (grouped in ≥34 246.0 ng/mL and <34 246.0 pg/mL), and for cardiovascular-related survival (C) by Hp level on admission (grouped in ≥177.1 ng/mL and <177.1 ng/mL) and (D) by NT-proBNP levels on admission (grouped in ≥11 848.5 ng/mL and <11 848.5 pg/mL).

indicate that Hp and NT-proBNP can be used to determine risk of survival in patients with acute HF. To our knowledge, this is the first study to demonstrate the prognostic value of these two markers in AHF.

Hp has been shown to be an independent biomarker in several diseases. A large clinical study by Holme *et al* found that elevated plasma Hp was a predictor for risk of cardiac disease risk in a health population.[26] They did not evaluate the prognostic value of Hp in patients with AMI. Brunetti *et al*[33] evaluated the effect of several acute phase proteins (APPs) on LV systolic function in early phase ST elevation MI (STEMI) and found that increased levels of APPs (including Hp) inversely correlated with LVEF among patients with STEMI. They concluded that such high levels may be linked with LV systolic dysfunction and a higher incidence of AHF.[33] However, a more recent study found that none of the APPs (including Hp) was consistently associated with atherosclerosis or plaque vulnerability.[34] On the other hand, the Malmo Preventive Project showed that increased concentrations of five inflammatory proteins (including Hp) were associated with myocardial infarction, stroke and other cardiovascular outcomes.[35 36]

By contrast, other studies have shown that low levels of Hp measured at the time of presentation in patients with AMI predicted the development of HF, indicating that the Hp level at the onset of an MI may be a prognostic biomarker of HF following AMI.[37] Their findings suggested that reduced levels of Hp could result in increased cardiac tissue damage during MI and, thus, would be associated with worse outcomes.[37] Consistent with this, a study by Arslan *et al*[38] showed that Hp deficiency resulted in impaired tissue repair and cardiac performance after MI. We also found lower Hp levels were associated with poorer outcomes (higher risk of death). The results of our study and prior studies suggest that an increased level of Hp may have a protective effect on the myocardium.

A cardiac protective effect by Hp was postulated to be related to its main function of binding and scavenging free Hb.[37] Hb binds and transports oxygen to the tissues. However, Hb is highly toxic when present in the free, unbound state in the blood.[37 39] Due to the iron bound to each heme group, free Hb leads to the formation of reactive oxygen species resulting in tissue damage.[37 40] Hp can bind to the free Hb and form a complex that is rapidly captured and degraded.[22 37] The binding of Hp to Hb serves to inhibit the oxidative potential of Hb by preventing the release of heme iron from Hb.[41–44]

Several additional models have been proposed to explain the protective effect of Hp in acute cardiovascular events. In an animal study, co-administration of Hp blocked the myocardial events induced by the combination of lipopolysaccharide and Hb.[45] This finding highlights the role of oxidative stress in mediating the development of atherosclerosis, that is, the oxidation hypothesis of atherosclerosis.[44 46] In this model, the most prominent target for oxidative modification is the LDL molecule. Oxidized LDL is pro-inflammatory and promotes vasoconstriction, monocyte adhesion, platelet aggregation and thrombosis.[44 47] Hp prevents oxidative stress on the vasculature exerted by Hb iron by forming the Hp-Hb complex whose clearance is mediated by the scavenger receptor CD163.[22 44]

Previous findings have implied that Hp genotype also plays a critical role in the oxidative and inflammatory response to intraplaque hemorrhage.[48] An in vitro study showed Hp 1–1 protein was superior to the Hp 2–2 protein in blocking the oxidative action of Hb.[42] In Hp 2–2 mice, atherosclerotic plaques contained more iron, lipid peroxidation and macrophage accumulation as compared with plaques from Hp 1–1 mice. In many human studies, the Hp 2–2 phenotype was found to be at significant risk for developing myocardial infarction, stroke and cardiovascular death,[26 49 50] while Hp 1–1 was associated with a strong protective effect regarding the primary end points (ie, death and HF) among patients with diabetes presenting with AMI.[51] Studies have shown that Hp 2–2-Hb complexes are cleared less efficiently than non-Hp 2–2-Hb complexes.[41 42 44] In patients with type 2 diabetes, this phenomenon is more pronounced due to the downregulation of CD163, particularly in Hp 2–2 individuals.[44 52] We did not evaluate the association of Hp phenotype with survival in this study.

In the current study, we found that NT-proBNP was also a prognostic indicator of survival. O'Brien *et al* studied acute LV failure in 34 patients admitted to the coronary care unit and found that predischarge NT-proBNP, but not admission NT-proBNP, predicted death or HF following AHF.[53] Since the present study did not measure the level of predischarge NT-proBNP, this may explain our discrepant results.

Our study had several limitations including its small cohort of patients (n=41) and the fact that it involved only a single center. In addition, the median duration of follow-up after initial presentation of AHF was 22 months, thus, less than one-third of the patients had completed follow-up after discharge at the time of the study. Therefore, we could not analyze the influence of either Hp or NT-proBNP levels after discharge on outcomes in our study cohort. In addition, the study only included severe patients who were NYHA III or IV. It was not possible to perform multivariable regression analysis since all non-survivors had Hp <177.1 ng/mL. Larger scale, more stringently designed studies are needed to confirm and extend our observations.

In conclusion, levels of Hp and NT-proBNP can predict survival in subjects with AHF.

Contributors D-YL: acquisition of data; analysis and interpretation of data; drafting of the manuscript; final approval of the manuscript; clinical studies. C-PL: conception and design; analysis and interpretation of data; drafting of the manuscript; experimental studies. T-MC: conception and design; analysis and interpretation of data; drafting of the manuscript; experimental studies. C-HW: acquisition of data; drafting of the manuscript; final approval of the manuscript; clinical studies. J-PP: conception and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; final approval of the manuscript; guarantor of integrity of the entire study; statistical analysis; definition of intellectual content; literature research; clinical studies; experimental studies; obtaining funding; administrative, technical or material support; supervision; critical revision of the manuscript.

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