ORIGINAL RESEARCH

The Additional Prognostic Value of Ghrelin for Mortality and Readmission in Elderly Patients with Acute Heart Failure

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Purpose: To evaluate the prognostic value of ghrelin, a growth hormone-releasing peptide, for mortality and readmission in elderly patients with acute heart failure (AHF).

Patients and Methods: We measured plasma ghrelin and pro B-type natriuretic peptide (NT-proBNP) levels upon emergency admission in 241 prospectively recruited elderly AHF patients (61.0% men). The outcomes were all-cause mortality and/or readmission due to heart failure (HF). Multivariate Cox proportional hazards regression analyses were used to evaluate the prognostic value of ghrelin. Discrimination, calibration, and reclassification indices were compared between models, with or without ghrelin.

Results: During 1.2 years of follow-up, we observed 90 events (57 deaths and 33 readmissions due to HF). Plasma ghrelin levels were significantly elevated in elderly AHF patients, when compared to healthy control subjects (P < 0.001). Patients with events had significantly higher baseline ghrelin levels, when compared to those without (P < 0.001). Ghrelin levels were positively correlated with NT-proBNP levels and HF severity, whereas they were negatively correlated with nutritional status (all P < 0.05). Log transformed ghrelin levels were independently associated with AHF events (hazard ratio = 2.64, 95% confidence interval = 1.11–6.25, P = 0.028). The incorporation of ghrelin into the reference model, or reference with the NT-proBNP model, both improved C-statistics (from 0.742–0.780 and 0.836–0.857; P = 0.074 and 0.044, respectively), resulting in an improvement in net reclassification index (14.42% and 10.45%, P = 0.020 and 0.025, respectively), and integrated discrimination index (5.64% and 3.60%, both P < 0.001). Patients who displayed the above NT-proBNP and ghrelin median levels had a markedly higher risk of AHF adverse events (P < 0.001).

Conclusion: Plasma ghrelin is an independent predictor of adverse events in elderly AHF patients. Ghrelin may provide additional value to clinical parameters or NT-proBNP for prognostic risk stratification in AHF.

Keywords: ghrelin, acute heart failure, prognosis, elderly

Introduction

Globally, heart failure (HF) represents a growing health burden, particularly in countries with a rapidly aging population.[1] Acute HF (AHF) patients are at considerably higher risk of morbidity and mortality when compared to chronic HF (CHF), with a one-year mortality rate of up to 30%, with approximately 20% of discharged AHF patients requiring readmission within 30 days.[2] Prognostic factors for AHF may differ from those used routinely for stable CHF.[3] The early identification of risk factors for vulnerable AHF patients can improve prognosis and aid

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decision making. While B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP) have emerged as robust diagnostic and prognostic AHF biomarkers, they should be cautiously interpreted in the elderly for their intrinsic associations with increasing age.[4] A combination with novel biomarkers and BNPs has markedly improved the ability to predict AHF prognosis.[5–7] However, the best biomarker combination has not yet been established, and most likely depends on clinical setting and population.

Ghrelin is a gut-derived 28-amino acid peptide that acts as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), which primarily participates in food intake and anabolism.[8] The cardiovascular protective effects of ghrelin have been recently suggested, and are mediated through both GH-dependent and independent mechanisms, providing improvements in energy balance, regulation of autonomic nervous activity, and direct effects on the heart and blood vessels.[9,10] Exogenous ghrelin administration improves cardiac function, decreases muscle wasting and enhances exercise capacity, suggesting this peptide can serve as a compensatory mechanism in response to anabolic-catabolic imbalance, under HF conditions.[11] However, the prognostic value of ghrelin has not been thoroughly studied, especially for AHF.

Both the incidence and prevalence of HF increase with age.[12] Older AHF patients are more prone to frailty, comorbidities, and impaired nutritional status that can impact HF outcomes.[13,14] Thus, in the present study, we aimed to investigate plasma ghrelin levels in elderly patients with AHF, and evaluate their prognostic value for adverse AHF events. We hypothesized that ghrelin would exert additional value in improving prognostic risk classifications in older AHF patients.

Patients and Methods
Study Population
We initially enrolled 293 patients aged ≥60, who were consecutively admitted with AHF as a first occurrence, or acute decompensation of CHF to the emergency department of Fujian Provincial Hospital, between December 2016 and June 2017. Of these, 52 were excluded; 26 due to incomplete data, eight for malignant tumors with expected survival time < 6 months, seven for a history of gastrointestinal ulcer or subtotal gastrectomy, two for endocrine or exocrine pancreatic disease, and nine for severe infections or multiple organ dysfunction

syndrome. Thus, 241 subjects were included. We also included 65 age-matched control subjects from the health-care center of Fujian Provincial Hospital. Controls had no acute or chronic disease, or were taking any regular medication. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Fujian Provincial Hospital. Written informed consent was obtained from all participants following a detailed description of the potential study benefits and risks.

Data Collection
Patient demographics, lifestyle habits, medical history, vital signs, nutritional status, physical examinations, laboratory tests and echocardiography data were collected upon admission to the emergency department. AHF stages were graded into class I, warm-dry; class II, warm-wet; class-III, cold-dry; class-IV, cold-wet, according to the "Bedside Clinical Classification" from the 2016 European Society of Cardiology (ESC) HF Guidelines.[4] Patients were classified as heart failure with preserved ejection fraction (HFpEF; left ventricular EF ≥ 50%), HF with reduced EF (HFrEF; LVEF < 40%), and HF with mid-range EF (HFmrEF; LVEF between 40% and 49%).[4] Cachexia was defined by edema-free weight loss of at least 5% (or BMI < 20kg/m^2) in 12 months or less in the presence of underlying illness, plus three of the following criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass, and abnormal biochemistry (increased inflammatory markers, anemia, low serum albumin).[15]

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), hemoglobin, albumin, prealbumin, creatinine, blood urea nitrogen (BUN), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), instant blood glucose, serum sodium, NT-proBNP, and cardiac troponin I (cTnI) were analyzed. Estimated glomerular filtration rate (eGFR) was calculated using the MDRD formula.[16] Echocardiography (General Electric Medical Systems, Vivid E9, Milwaukee, WI, USA) was used to measure left atrial dimension (LAD), left ventricular end diastolic diameter (LVEDD), and left ventricular ejection fraction (LVEF). Nutritional status was evaluated by Mini Nutritional Assessment Short Form (MNA-SF).[17] Risk of malnutrition was defined according to MNA-SF criteria as <12 points.

Plasma total ghrelin levels were measured by a commercial enzyme immunoassay (EIA) kit (Phoenix

Pharmaceuticals Inc., Burlingame, CA, USA). Blood samples were drawn at four hours after a meal, and plasma was separated in a chilled centrifuge for 15 min at 3000 rpm, then immediately transferred into 1 mL cryotubes and stored at −80°C until required. Intra- and inter-assay coefficients of variation (CV) for EIA were 4.6% and 8.1%, respectively.

Follow-Up and Events

Follow-up was conducted during regular outpatient visits or telephone calls. The study outcome was the composite event of all-cause mortality and/or readmission due to HF. Medical records were reviewed to confirm information. All patients received optimal medication tailored to HF or other comorbidities (eg, diabetes, hypertension, dyslipidemia) during follow-up.

Statistical Analyses

Clinical data from control subjects and AHF patients, or patients with or without events, were compared using the Student's *t* test for variables with normal distribution, the Mann–Whitney *U*-test for variables with skewed distribution, and the Chi-square test for categorical parameters. Correlations between ghrelin and other variables were examined by Pearson's or Spearman's Rank correlation analysis.

Cox proportional hazard models were constructed to explore relationships between independent predictors and outcomes. Because ghrelin and NT-proBNP levels had skewed distributions, they were logarithmically transformed to generate linear trends, and to minimize the influence of outliers. Linear relationships between independent variables and outcomes were appropriate, and no multi-collinearity existed between covariates. Proportional hazard assumptions were met, and interaction terms were not significant, and were therefore disregarded in final models. After making adjustments for confounding factors, we calculated the hazard ratios (HRs) and 95% confidence interval (CI) of AHF events for ghrelin and NT-proBNP levels. We constructed penalized cubic spline curves to evaluate the shape of associations between NT-proBNP and ghrelin levels and AHF events.

Traditional variables considered clinically relevant or showing univariate associations (P < 0.1) with outcomes, were entered into multivariate Cox proportional hazards regression analyses to build a reference model. Variables for inclusion were carefully chosen, given the number of events available, to ensure parsimony of the final model.

The Cox regression model, with a backward stepwise selection method, was used to determine independent predictors for outcomes. The prognostic value of ghrelin to improve risk predictions on top of the reference model was evaluated. The discriminative ability of each model was evaluated by the C-statistic, identical to the area under the receiver operating characteristics (ROC) curve (AUC). DeLong tests were used to compare the C-statistics of each model.[18] Calibration was evaluated by the Hosmer-Lemeshow goodness of fit test. To calculate the proportion of patients who were reclassified by advanced models, when compared with the reference model, we computed the net reclassification index (NRI) and the integrated discrimination index (IDI). As stated by previous articles,[6,19] patients were regarded at high-risk if the predicted risk of adverse events was approximately two-fold of the observed, whereas a predicted risk around half of the observed was considered to be a low-risk category. Corresponding cut-offs for predicted AHF events risk were <30%, ≥30% to <60%, and ≥60%. Cumulative event-free probability was demonstrated by Kaplan-Meier curves, and compared using log rank tests. Statistical analyses were performed using Stata 15.1, MedCalc, and R 3.5.0 statistical software. A two-sided P value <0.05 was considered statistically significant.

Results

Two hundred and forty-one elderly patients with AHF were included (mean age = 68.6 years, 61.0% men). There were 135 patients were classified as HFpEF, 65 patients as HFrEF, and 41 as HfmrEF. Two patients with HFrEF were on implantable cardioverter defibrillator (ICD) therapy. Thirty-nine patients were identified with cachexia. During 1.2 years of follow-up (median follow-up duration = 446 days), there were 90 events (57 deaths and 33 readmissions due to HF). The baseline characteristics of study participants are shown (Table 1). Patients with events had lower diastolic blood pressure (DBP), body mass index (BMI), MNA-SF scores, albumin, pre-albumin, hemoglobin, eGFR, TC, LDL, serum sodium, whereas they had higher respiratory rates (RR), creatinine, BUN, NT-proBNP, ghrelin and a more severe HF stage. They were more likely to have a history of HF hospitalization, and present AHF as decompensated CHF, than as a first occurrence (all P < 0.05). AHF patient characteristics and control subjects are summarized (Table S1). We observed no significant differences in age, sex, BMI, systolic blood pressure (SBP), DBP between the two groups.

Table 1 Baseline Characteristics of Elderly AHF Patients

Variables	No Events (N = 151)	Events (N = 90)	P value
General Characteristics			
Age (year)	67.28 ± 13.27	70.78 ± 14.74	0.059
Male (N, %)	58 (38.4%)	36 (40.0%)	0.89
Smoking (N, %)	65 (43.0%)	38 (42.2%)	0.99
Alcohol (N, %)	24 (15.9%)	15 (16.7%)	0.86
Physical Exam			
SBP (mmHg)	128.5 (114, 151)	128 (109, 145)	0.32
DBP (mmHg)	79 (69, 90)	74 (65, 85)	**0.04**
BMI (kg/m^2)	23.60 (20.68, 25.83)	21.68 (18.95, 25.31)	**0.037**
Heart rate (bpm)	88.5 (74, 104)	90 (80, 106)	0.18
RR (breaths/min)	20 (18, 22)	22 (20, 25)	**<0.001**
MNA-SF score	11 (8, 13)	9 (6, 12)	**0.009**
Laboratory data			
ALT (U/L)	26 (18, 49)	27.5 (17, 50)	0.73
AST (U/L)	34 (21, 100)	35 (23, 66)	0.95
Albumin(g/L)	37 (34, 40)	36 (30, 39)	**0.028**
Prealbumin(mg/L)	177.7 (128.1, 207.0)	144.1 (104.1, 189.9)	**0.025**
Hemoglobin (g/L)	137 (118, 150)	126 (99, 145)	**0.003**
Creatinine (μmol/L)	82.5 (69, 116)	105.5 (82, 140)	**<0.001**
BUN (mmol/L)	6.5 (4.8, 8.4)	8.85 (6.0, 12.5)	**<0.001**
eGFR (mL/min)	74.37 (52.40, 100.85)	57.01 (40.12, 75.59)	**<0.001**
TG (mmol/L)	1.27 (0.99, 1.84)	1.19 (0.84, 1.63)	0.14
TC (mmol/L)	4.19 (3.57, 4.99)	3.75 (2.97, 4.73)	**0.009**
HDL-C (mmol/L)	1.02 ± 0.36	0.99 ± 0.37	0.51
LDL-C (mmol/L)	2.63 (2.16, 3.34)	2.19 (1.64, 3.03)	**0.003**
Instant blood glucose (mmol/L)	5.62 (4.78, 7.39)	5.42 (4.50, 6.86)	0.12
NT-proBNP (ng/mL)	3241 (1558, 7796)	8003 (2778, 15,136)	**<0.001**
Ghrelin (ng/mL)	0.97 (0.75, 1.20)	1.19 (1.07, 1.36)	**<0.001**
Serum sodium (mmol/L)	139 (136, 141)	137 (134, 140)	**0.014**
cTnI (ng/mL)	0.185 (0.03, 2.65)	0.13 (0.05, 0.96)	0.79
Echocardiographic data			
LAD (cm)	4.07 (3.59, 4.59)	4.14 (3.43, 4.61)	0.90
LVEDD (cm)	5.04 ± 0.87	5.16 ± 1.05	0.46
LVEF (%)	53 (44, 58)	50.5 (40, 58)	0.43
Comorbidities			
Hypertension (N, %)	93 (61.6%)	58 (64.4%)	0.68
Diabetes (N, %)	49 (32.5%)	30 (33.3%)	0.89
Dyslipidemia (N, %)	35 (23.2%)	16 (17.8%)	0.42
ASCVD (N, %)	52 (34.4%)	34 (37.8%)	0.68
CKD (N, %)	12 (7.9%)	13 (14.4%)	0.13
History of HF hospitalization (N, %)	54 (35.8%)	57 (63.3%)	**<0.001**
AHF stage			
Bedside Clinical Classification (III~IV) (N, %)	83 (55.0%)	65 (72.2%)	**0.009**
AHF type			
First occurrence (N, %)	61 (40.4%)	17 (18.9%)	**<0.001**
Decompensated CHF (N, %)	90 (59.6%)	73 (81.1%)	**<0.001**

(Continued)

Table 1 (Continued).

Variables	No Events (N = 151)	Events (N = 90)	P value
AHF cause			0.35
CHD (N, %)	77 (51.0%)	42 (46.7%)	
Hypertension (N, %)	25 (16.6%)	21 (23.3%)	
DCM (N, %)	14 (9.3%)	12 (13.3%)	
Valvular heart disease (N, %)	12 (7.9%)	3 (3.3%)	
Others (N, %)	23 (15.2%)	12 (13.3%)	

Note: Continuous variables were reported as mean ± deviation (SD) or median (upper and lower quartile), and categorical variables as percentages (%). P value < 0.05 is shown in bold type.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; RR, respiratory rate; MNA-SF, Mini Nutritional Assessment Short Form; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein protein cholesterol; cTnI, cardiac troponin I; LAD, left atrial dimension; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; ASCVD, atherosclerotic cardiovascular disease; CKD, chronic kidney disease; CHF, chronic heart failure; AHF, acute heart failure; CHD, coronary heart disease; DCM, dilated cardiomyopathy.

Plasma ghrelin levels were significantly higher in AHF patients than in control subjects (P < 0.001).

After log transformation, ghrelin levels were positively associated with NT-proBNP levels (Pearson's r = 0.2323, P = 0.0003, Figure 1A), and negatively associated with MNA-SF scores (Pearson's r = −0.1865, P = 0.0037, Figure 1B). Ghrelin levels were also correlated with HF severity as assessed by clinical bedside classification (Spearman's r = 0.1349, P = 0.037).

Cox proportional hazard regression revealed that log NT-proBNP and log ghrelin levels were associated with AHF events, after adjustment for covariates (HRs = 2.17 and 2.64, P < 0.001 and P = 0.028, respectively, Table S2). Penalized cubic spline curves demonstrated graded increases for the risk of adverse AHF events, with increasing log NT-proBNP and log ghrelin levels after adjusting for the same confounding factors (Figure 2).

The C-statistics for the reference model (including RR, eGFR, serum sodium, and history of HF hospitalization) were 0.742 (95% CI: 0.682–0.796) for AHF events, which showed an improvement tendency with the addition of log transformed NT-proBNP or ghrelin levels (ΔC-statistics = 0.094 and 0.038, P for improvement = 0.007 and 0.074, respectively). The highest C-statistics were observed in the dual addition model of log NT-proBNP and log ghrelin (C-statistics = 0.857, 95% CI: 0.806–0.898, Figure 3 and Table 2). Calibration plots for AHF events were built for each model, and all performed well (all Hosmer-Lemeshow χ^2 < 20, all P > 0.05, Figure S1 and Table S3). As shown (Tables 3 and 4), the addition of log ghrelin to the reference model, or to the reference model with log NT-proBNP, both improved the risk classification, with an NRI value of 14.42% and 10.45% (P = 0.020 and 0.025), respectively.
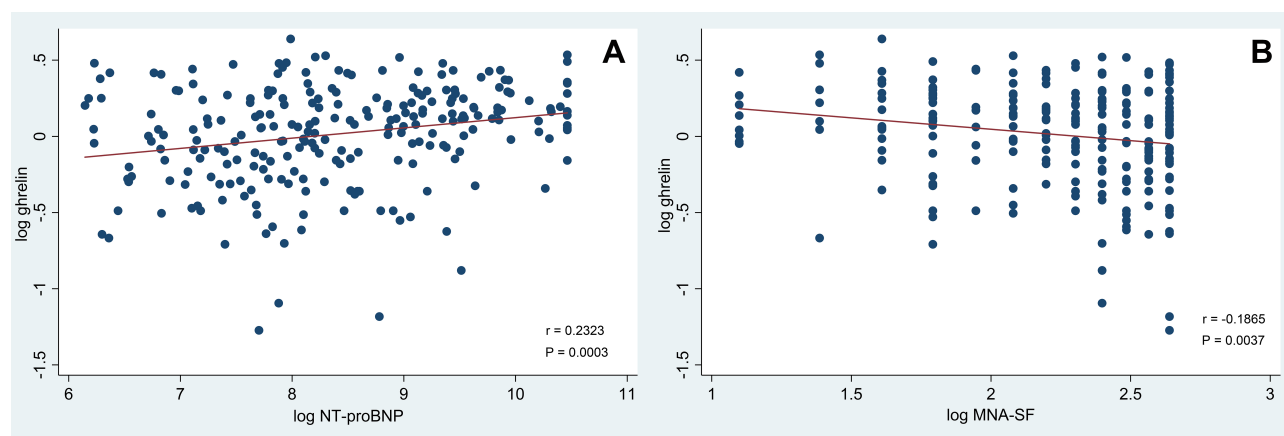

Figure 1 Association of ghrelin levels with (**A**) NT-proBNP levels and (**B**) MNA-SF scores.
Note: Pearson's correlation analysis for the association of log ghrelin with log NT-proBNP levels (**A**), and log MNA-SF scores (**B**).
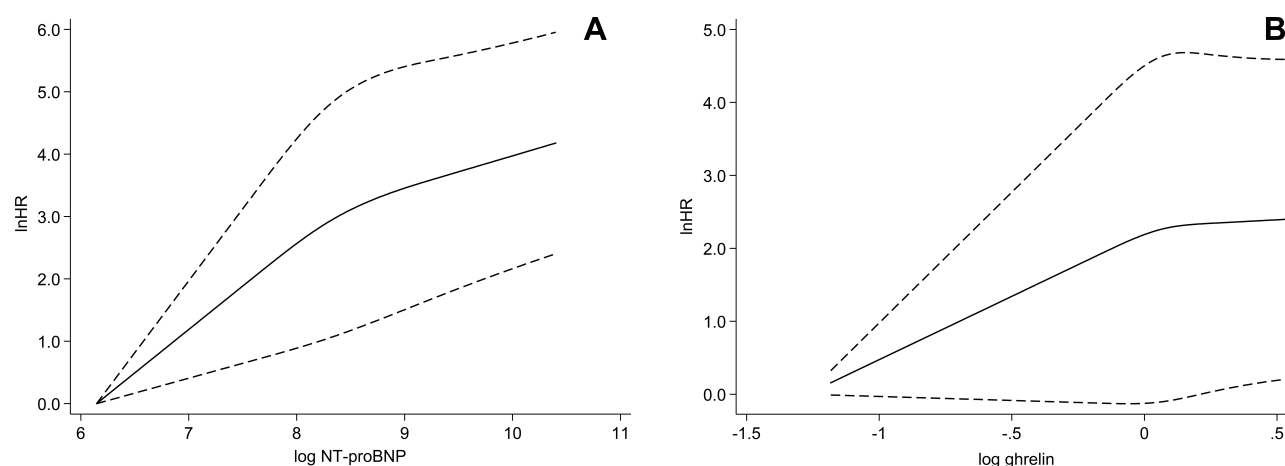**Abbreviation:** MNA-SF, Mini Nutritional Assessment Short Form.

Figure 2 Association of NT-proBNP and ghrelin levels with acute heart failure events.
Note: Penalized cubic spline curves based on the Cox regression model (adjusted by age, sex, smoking status, BMI, RR, heart rate, SBP, DBP, hemoglobin, eGFR, bedside clinical classification, MNA-SF score, serum sodium, and history of HF hospitalization) were used to examine the association of log transformed NT-proBNP (**A**) and ghrelin (**B**) levels with AHF adverse events. Solid lines indicate lnHRs, and dashed lines indicate 95% confidence intervals (CI).
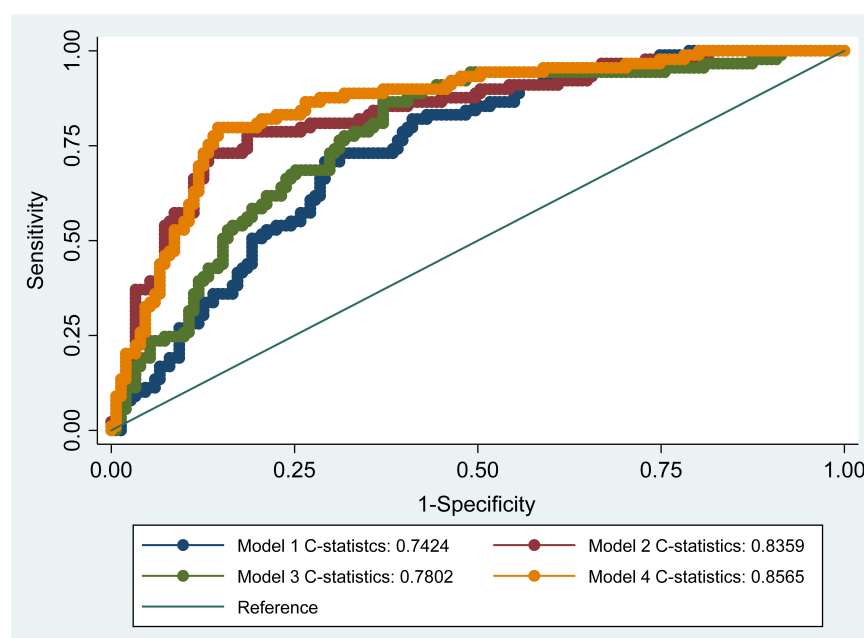

Figure 3 ROC curves of each model for acute heart failure events.
Note: Model 1 (reference) includes RR, eGFR, serum sodium, and history of HF hospitalization; Model 2, Model 1 + log NT-proBNP; Model 3, Model 1 + log ghrelin; Model 4, Model 1 + log NT-proBNP + log ghrelin.

Similarly, significant improvements in IDI were noted (5.64% and 3.60%, both P < 0.001).

According to the dual addition prediction model of log transformed NT-proBNP and ghrelin, AHF patients were categorized into three risk groups (<30%, ≥30% to <60%, and ≥60%). Kaplan-Meier curves showed significant differences in risks for AHF events between groups (log rank test, P < 0.001, Figure 4). We further classified patients into other groups, based on NT-proBNP and ghrelin levels above or below the median; Kaplan–Meier curves showed that patients with higher NT-proBNP and ghrelin levels were at considerably higher risk of AHF events (log rank test, P < 0.001, Figure 5).

Discussion

In this study, we investigated the additional prognostic value of ghrelin, a growth hormone–releasing peptide, in elderly AHF patients. Our main finding was that ghrelin has the

Table 2 C-Statistic Comparisons Between Models

Variables	ΔC-Statistics	SD	P value
Model 1 vs 2	0.0935	0.0296	0.0070
Model 1 vs 3	0.0378	0.0212	0.0744
Model 2 vs 4	0.0206	0.0102	0.0439
Model 3 vs 4	0.0763	0.0229	0.0009

Note: Model 1 (reference) included respiratory rate, eGFR, serum sodium, and history of HF hospitalization. Model 2, Model 1 + log NT-proBNP; Model 3, Model 1 + log ghrelin; Model 4, Model 1 + log NT-proBNP + log ghrelin. DeLong test was used to compare the C-statistics of each model.
Abbreviation: SD, standard deviation.

Table 3 Risk Reclassification with Ghrelin Addition

Predicted risk Reference model	Reference model + log ghrelin			
	< 30%	≥ 30% to < 60%	≥ 60%	Total
Patients who did not experience AHF events				
< 30%	84	11	0	95
≥ 30% to < 60%	17	30	4	51
≥ 60%	1	2	2	5
Total	102	43	6	151
Patients who experienced AHF events				
< 30%	16	8	0	24
≥ 30% to < 60%	4	46	7	57
≥ 60%	0	1	8	9
Total	20	55	15	90

NRI (95% CI): 14.42% (2.25%, 26.6%), P = 0.02026;
NRI (no events): 3.31%, NRI (events): 11.11%;
IDI (95% CI): 5.64% (3.18%, 8.11%), P = 0.00001.

Notes: Reclassification tables are separated for absent AHF events and present AHF events, with rows indicating risk categories based on the reference model (RR, eGFR, serum sodium, and history of HF hospitalization). Columns indicate new risk stratifications after adding log ghrelin to the reference model, or reference model with log NT-proBNP. Green numbers reflect patients who changed risk category in a beneficial direction by adding log ghrelin, while blue numbers reflect patients reclassified in the inappropriate direction. Patients in grey boxes have the same predicted risk with both models. AHF events: composite events of all-cause mortality and/or readmissions due to HF.
Abbreviations: NRI, net reclassification index; IDI, integrated discrimination index; AHF, acute heart failure.

Table 4 Risk Reclassification with Dual Addition of NT-proBNP and Ghrelin

Predicted risk Reference model + log NT-proBNP	Reference model + log NT-proBNP + log ghrelin			
	< 30%	≥ 30% to < 60%	≥ 60%	Total
Patients who did not experience AHF events				
< 30%	114	5	0	119
≥ 30% to < 60%	6	15	2	23
≥ 60%	0	0	9	9
Total	120	20	11	151
Patients who experienced AHF events				
< 30%	15	4	0	19
≥ 30% to < 60%	1	27	8	36
≥ 60%	0	1	34	35
Total	16	32	42	90

NRI (95% CI): 10.45% (1.34%, 19.56%), P value: 0.0246;
NRI (no events): -0.0067%; NRI (events): 11.11%;
IDI (95% CI): 3.60% (1.59%, 5.60%), P value = 0.00043.

Notes: Reclassification tables are separated for absent AHF events and present AHF events, with rows indicating risk categories based on the reference model (RR, eGFR, serum sodium, and history of HF hospitalization). Columns indicate new risk stratifications after adding log ghrelin to the reference model, or reference model with log NT-proBNP. Green numbers reflect patients who changed risk category in a beneficial direction by adding log ghrelin, while blue numbers reflect patients reclassified in the inappropriate direction. Patients in grey boxes have the same predicted risk with both models. AHF events: composite events of all-cause mortality and/or readmissions due to HF.
Abbreviations: NRI, net reclassification index; IDI, integrated discrimination index; AHF, acute heart failure.

potential to improve predictions for mortality and readmissions due to HF, beyond a model with established clinical variables. Furthermore, combined NT-proBNP and ghrelin provided an incremental added value over NT-proBNP alone. We demonstrated for the first time, that plasma ghrelin can be used as a prognostic biomarker for adverse event incidences in elderly AHF patients.

Ghrelin levels were elevated in elderly AHF patients, when compared with control subjects, and were positively correlated with NT-proBNP levels and HF severity of HF as assessed by clinical bed classification. These results were generally consistent with previous studies investigating AHF and CHF.[20–23] It has been reported that acute increases in ghrelin do not interfere with cardiac metabolism in normal individuals, whereas they enhance free fatty acid oxidation and reduce glucose oxidation in HF, thus partially correcting metabolic alterations during AHF.[24] Ghrelin also reduces mean arterial blood pressure, increases myocardial contractility, inhibits cardiomyocyte and endothelial cell apoptosis, and decreases sympathetic nerve activity via AMPK, AKT, ERK1/2, and GSK3 α/β pathways,[25] all of which play beneficial roles in early HF stages.[10,11,25] However, Chen et al[26] showed that plasma ghrelin levels were significantly lower in CHF patients. Similarly, another study claimed that ghrelin expression in myocardial biopsies from CHF patients undergoing heart transplantation was significantly decreased at the atrium and ventricles.[27] We speculate this disparity may be explained by the HF stage. Elevated ghrelin in AHF patients can be a protective compensatory mechanism. With HF progression, endogenous ghrelin is inadequate in reversing or improving HF, leading to ghrelin depletion and HF decompensation. This hypothesis is supported by
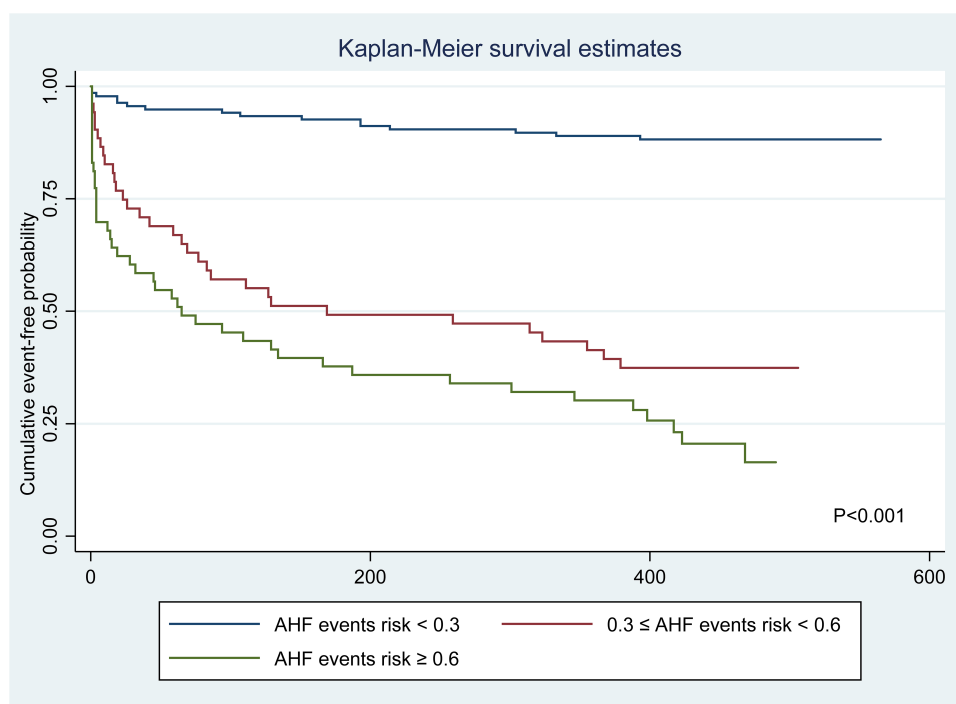
Figure 4 Kaplan–Meier curves of AHF event risks according to models with dual addition of log ghrelin and log NT-proBNP.
Note: AHF events: all-cause mortality and/or readmission due to heart failure.
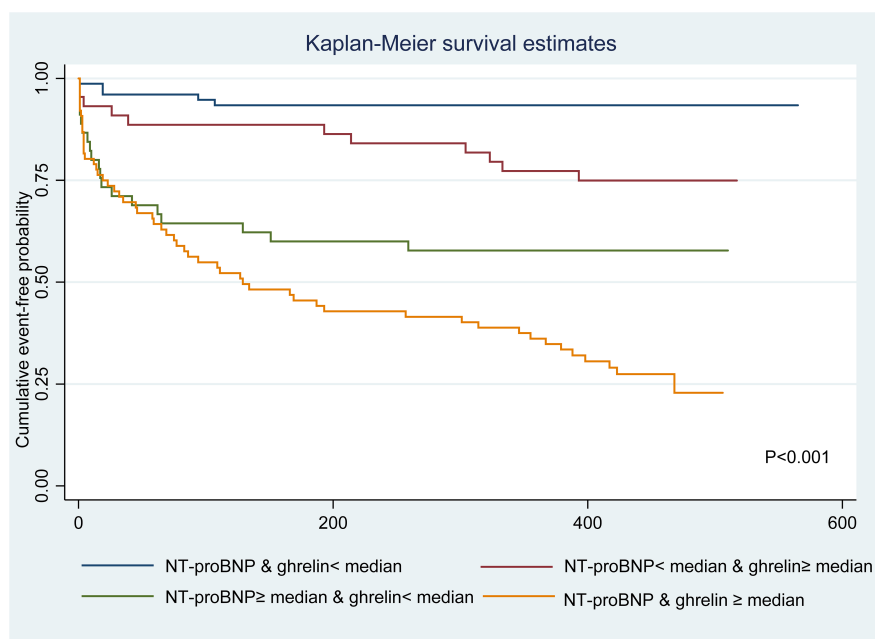**Abbreviation:** AHF, acute heart failure.

Figure 5 Kaplan–Meier curves of AHF events risk according to NT-proBNP and ghrelin levels.
Note: NT-proBNP median level: 3980ng/mL, ghrelin median level: 1.07ng/mL. AHF events: all-cause mortality and/or readmission due to heart failure. Cumulative event-free probability was compared by log rank test.
Abbreviation: AHF, acute heart failure.

findings that ghrelin is higher in patients with more recent diagnoses when compared with patients with prolonged dilated cardiomyopathy (DCM).[28] Ghrelin levels are elevated in response to negative energy balances in elderly AHF patients, to reverse metabolic alterations in HF. Itoh et al observed increased ghrelin levels in underweight

patients with chronic obstructive pulmonary disease,[29] which may involve similar mechanisms.

There is little evidence for the prognostic role of ghrelin in HF. In an in vitro study,[30] ghrelin-treated DCM mice had significantly prolonged life spans, when compared with saline-treated control DCM mice. Chen et al[26] suggested that low ghrelin levels were independently associated with adverse cardiac events in CHF patients. In this study, we observed that for elderly AHF patients, increased ghrelin levels had additional prognostic values for mortality and readmission due to HF, over clinical variables and NT-proBNP levels. Elevated ghrelin levels directly decreased blood pressure, and inhibited sympathetic drive in AHF patients, which was related to AHF severity, potentially indicating a poor HF prognosis.[20,23,31] Incremental ghrelin levels are not only associated with cardio-protective effects via GH-dependent or independent pathways, but are also attributed to their correlation with nutritional status. Risk of malnutrition has been implicated in HF development and progression.[32] Indeed, we observed a high prevalence of risk of malnutrition (62.24%) in our AHF patients. We also found patients with events had lower albumin, prealbumin and hemoglobin levels, BMI and MNA-SF scores, than patients free of events. It was reported that nutritional status appears to be a major determinant of ghrelin release from the stomach.[9] This relationship is supported by our study, where ghrelin levels were negatively correlated with MNA-SF scores. The most severe form of malnutrition is cardiac cachexia. Circulating ghrelin levels are elevated in CHF patients with cachexia, reflecting a negative energy balance state.[33] Although this underlying mechanism is not clear, it may represent a physiological compensation to increase appetite and caloric intake, thus maintaining an anabolic-catabolic balance.[22]

C-statistics for AHF adverse events prediction were significantly improved upon the addition of NT-proBNP, but when ghrelin was added, they did not reach statistical significance. We believe this can be explained by the properties of C-statistics. They barely change when good risk factors are included in the model, and therefore they require large HRs to achieve statistical significance.[34] Although NT-proBNP was more strongly associated with adverse events than ghrelin, their combination improved the risk classification of AHF events, than NT-proBNP alone. It is accepted that BNP levels are associated with a variety of cardiac and non-cardiac factors, such as advanced age, impairments in renal clearance, atrial and ventricular tachyarrhythmias and anemia.[4] The diagnostic and prognostic ability of BNPs may be limited in situations where elderly patients with renal dysfunction and advanced HF, already have high BNP levels. At present, improved risk stratifications of adverse events in AHF patients may be derived by combining several biomarkers and clinical variables, but not a single biomarker. NT-proBNP and ghrelin likely reflect different pathophysiological mechanisms, responsible for alleviating AHF. In elderly AHF patients, ghrelin reflects different aspects of cardiovascular disease pathology, such as energy balance and appetite stimulation, that are not represented by established HF biomarkers related to HF outcomes. Accordingly, ghrelin addition may provide more incremental information beyond cardiac markers, such as BNPs.

This study generated several clinical insights. Firstly, ghrelin as an integrator of appetite and anabolic factors may provide promising therapeutic options for HF, especially in cardiac cachexia.[35] Nagaya et al demonstrated that exogenous ghrelin administration significantly decreased mean arterial pressure and systemic vascular resistance,[31] reduced plasma norepinephrine and BNP levels, increased LVEF, and improved muscle wasting in CHF patients (80% with cardiac cachexia).[11] However, ghrelin regulatory effects are complex. Based on existing information, we believe ghrelin levels fluctuate during HF progression, plus there may be a "ghrelin resistance" phenomenon existing in advanced HF.[22] Therefore, more evidence is required to determine the ideal ghrelin intervention point to optimally benefit HF patients. Equally, larger cohorts with longer follow-up durations are required to strengthen the prognostic significance of ghrelin in AHF patients. Secondly, ESC guidelines for treating AHF recommend monitoring body weight and preventing malnutrition in HF patients.[4] For the elderly, the age-related reduction in energy or protein intake increases malnutrition risks. Such nutritional deficiencies are both short- and long-term prognostic factors for AHF patients.[36,37] The risk of malnutrition should be considered a clinical factor in HF progression; thus, the early identification of and intervention for malnutrition could improve HF prognoses.[38] Thirdly, when considering the high prevalence of frailty and comorbidities, older patients should undergo multidimensional comprehensive geriatric assessments before discharge, to combat physical deconditioning and loss of independency.[39] Elderly patients differ substantially to younger patients in terms of AHF clinical characteristics; thus, the exploration of specific prognostic factors in this population is essential.

This study has clarified the previously unclear prognostic value of ghrelin for adverse events in elderly AHF patients.

However, we acknowledge some study limitations. Firstly, this was a single-center study and our population was small; however, numbers were comparable to other studies that investigated similar objectives and outcomes. Secondly, ghrelin levels were measured upon admission to the emergency department instead of after fasting; therefore, the effects of food on ghrelin levels cannot be disregarded. However, blood was drawn four hours after eating, which reduced some bias. Thirdly, we included an older subpopulation of AHF patients, which may have represented a cohort with more advanced HF; thus, extrapolations from our findings towards the AHF population as a whole, require further evaluation. Lastly, our NRI findings in the absence of an accepted risk grouping must be cautiously interpreted.

Conclusions

Plasma ghrelin levels are elevated in elderly AHF patients, and are associated with mortality and readmission due to HF. They provide an additive value to clinical parameters, or NT-proBNP for AHF risk stratification. Therefore, ghrelin represents a promising therapeutic target for HF.

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Disclosure

The authors report no conflicts of interest in this work.

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