

Serum retinol-binding protein 4 as a predictor of cardiovascular events in elderly patients with chronic heart failure

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Abstract

Aims RBP4 is an adipokine with adverse effects on cardiovascular system. Increased circulating retinol-binding protein 4 (RBP4) has been linked to chronic heart failure (CHF). However, whether elevated RBP4 is correlated with a poor prognosis in elderly patients with CHF remains unclear. The aim of this study was to evaluate the prognostic value of serum RBP4 in elderly patients with CHF.

Methods and results We enrolled 934 consecutive elderly patients (aged 60 years and older) with CHF and 138 age-matched and sex-matched control subjects in a prospective cohort study and explored the association of serum RBP4 levels with the clinical outcomes using multivariate Cox regression analyses. Serum RBP4 levels were elevated in CHF patients when compared with controls ($46.66 \pm 12.38 \mu\text{g/mL}$ vs. $40.71 \pm 7.2 \mu\text{g/mL}$, $P < 0.001$). Patients with the highest RBP4 concentrations had higher N terminal pro brain natriuretic peptide (NT-proBNP) levels but lower left ventricular ejection fraction (LVEF) and estimated glomerular filtration rate ($P < 0.001$). Serum RBP4 levels were increased as the New York Heart Association functional class increased and LVEF decreased ($P < 0.001$) and were negatively correlated with LVEF ($r = -0.154$, $P < 0.001$) but positively correlated with NT-proBNP levels ($r = 0.074$, $P = 0.023$). Multivariate Cox regression analysis suggested that log RBP4 was an independent predictor for major adverse cardiac event(s) [hazard ratio (HR) = 2.61, 95% confidence interval (CI) = 1.19–5.70], together with age, male, LVEF, log NT-proBNP, and estimated glomerular filtration rate. Moreover, log RBP4 was also an independent predictor for cardiovascular mortality (HR = 2.24, 95% CI = 1.35–5.39) and CHF rehospitalization (HR = 2.54, 95% CI = 1.09–5.60) even after adjustment for the established adverse prognostic factors for CHF. The Kaplan–Meier survival curves showed that high concentration of RBP4 was a prognostic indicator of major adverse cardiac event(s) in patients with CHF.

Conclusions Our findings demonstrate for the first time that elevated serum RBP4 is correlated with worse outcome in elderly patients with CHF.

Keywords Chronic heart failure; Elderly; Retinol-binding protein 4; Major adverse cardiac events

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Introduction

Chronic heart failure (CHF) is a major cause of hospitalization in the elderly, leading to a high risk of mortality,

disability, and rehospitalization.¹ Due to poor prognosis and high costs of therapy, adequate risk assessments and optimized medical treatment are essential in elderly patients with CHF.²

Retinol-binding protein 4 (RBP4) is an approximately 21-kDa secreted protein that mediates the transport of vitamin A (retinol) in circulation.³ RBP4 has been well known as an important adipokine that contributes to insulin resistance and obesity.^{4,5} Recent clinical studies have also linked higher circulating RBP4 to various cardiovascular diseases.⁶ Serum RBP4 levels were found to be increased in patients with advanced heart failure and correlated with insulin resistance.⁷ Another clinical study showed that plasma RBP4 was increased in patients with inflammatory cardiomyopathy.⁸ Our previous study demonstrated that RBP4 could promote cardiac hypertrophy via inducing proinflammatory responses in cardiomyocytes through Toll-like receptor 4/myeloid differentiation primary response gene 88 pathway.⁹ However, whether elevated RBP4 is correlated with a poor prognosis in patients with CHF remains unclear. Therefore, we carried out a prospective cohort study to evaluate the prognostic value of serum RBP4 in elderly patients with CHF.

Methods

Study population

A total of 934 consecutive elderly patients (aged 60 years and older) with CHF admitted to the affiliated hospitals of Nanjing Medical University were recruited between 1 October 2010 and 31 July 2013. The control subjects were selected during the same period in the same hospital from the health examination centre. This study was performed in accordance with the principles outlined in the *Declaration of Helsinki*¹⁰ and approved by the Ethics Committee of Nanjing Medical University. The diagnosis of CHF was on the basis of typical symptoms and signs and evidence of left ventricular diastolic and/or systolic dysfunction, according to the American College of Cardiology/American Heart Association guidelines.¹¹ All patients had a history of CHF for at least 6 months and were in stable condition on medication for at least 1 month before blood sampling. All subjects included in this study had no history of significant concomitant diseases, including severe hepatic or renal diseases, bleeding disorders, previous thoracic irradiation therapy, autoimmune disease, and malignant diseases. All patients received standard medical treatment, and written informed consent was obtained from each participant.

Serum RBP4 measurements

Serum RBP4 levels were assayed in duplicate by using a sandwich enzyme-linked immunosorbent assay kit (R&D, Minneapolis, MN, USA) according to the manufacturer's protocol. The intra-assay and inter-assay coefficients of variance were

2.32% and 2.95%, respectively. The analytic sensitivity of the assays was 0.021 ng/mL.

Clinical outcomes

The primary clinical outcome was major adverse cardiac event(s) (MACE), which was defined as cardiovascular death and rehospitalization due to worsening CHF. The secondary endpoints were the individual components of the primary outcome, including cardiovascular mortality and CHF rehospitalization. Endpoints were obtained by reviewing the hospital database and by contacting each patient individually.

Statistical analysis

Normality of distribution was assessed using the Kolmogorov–Smirnov test. RBP4 were normally distributed parameters and presented as the mean \pm standard deviation. Skewed data were expressed as median and quartile ranges, and comparisons were analysed by the Mann–Whitney *U* test. Pearson χ^2 test was used to compare qualitative variables represented as frequencies. The correlations between serum RBP4 levels and cardiac function variables were calculated using Spearman correlation coefficient. The association between baseline variables and MACE was evaluated using univariable and multivariable Cox proportional hazards analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. The factors entered into the Cox regression model were age, sex, body mass index (BMI), ischaemic aetiology, hypertension, diabetes, smoking, New York Heart Association (NYHA) functional class, left ventricular ejection fraction (LVEF), N terminal pro brain natriuretic peptide (NT-proBNP), estimated glomerular filtration rate (eGFR), medical treatments, and RBP4. Kaplan–Meier analysis was conducted to compare the differences of survival rates between patients with high and low levels of RBP4 using the log-rank test. All tests were two sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using PASW 18.0 (IBM SPSS, Inc., Chicago, USA).

Results

Baseline characteristics

The baseline characteristics of the participants are shown in *Table 1*. There were no statistically significant differences between CHF patients and control subjects with respect to age, proportion of male, BMI, rates of smoking, hypertension and diabetes, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. However, CHF

Table 1 Baseline characteristics of the control subjects and elderly patients with CHF

Variables	Control (n = 138)	CHF (n = 934)	P value
Age (years)	69 (64–76)	68 (66–74)	0.257
Male, n (%)	96 (69.6)	611 (65.4)	0.337
BMI (kg/m ²)	24.51 (22.66–27.00)	24.68 (22.49–26.67)	0.886
Smoking, n (%)	54 (39.1)	411 (44.0)	0.281
Hypertension, n (%)	60 (43.5)	392 (42.0)	0.782
Diabetes, n (%)	50 (36.2)	402 (43.0)	0.131
eGFR (mL/min/1.73 m ²)	104 (96–117)	75 (64–82)	<0.001
TC (mmol/L)	4.01 (3.39–5.04)	4.59 (3.90–5.20)	<0.001
TG (mmol/L)	1.25 (0.93–1.76)	1.50 (1.07–2.04)	<0.001
HDL-C (mmol/L)	1.04 (0.88–1.18)	1.07 (0.91–1.24)	0.057
LDL-C (mmol/L)	2.63 (2.15–3.40)	2.68 (2.26–3.28)	0.595
RBP4 (µg/mL)	40.71 ± 7.28	46.66 ± 12.38	<0.001

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RBP4, retinol-binding protein 4; TC, total cholesterol; TG, triglyceride.

RBP4 is presented as mean ± standard deviation. Other data are presented as median with interquartile range or number with percentage in parentheses.

patients had lower levels of eGFR and higher levels of total cholesterol and triglyceride ($P < 0.001$). In addition, serum RBP4 levels were increased in CHF patients when compared with the control subjects ($46.66 \pm 12.38 \mu\text{g/mL}$ vs. $40.71 \pm 7.2 \mu\text{g/mL}$, $P < 0.001$). We further divided the elderly patients with CHF into four subgroups according to the quartile values of serum RBP4 (Table 2). We found that patients with the highest RBP4 concentrations had higher NT-

proBNP levels but lower LVEF and eGFR ($P < 0.001$), as well as lower rates of medical treatments, including diuretics, spironolactone, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and beta-blockers ($P < 0.05$). The median length of follow-up was 736 days (range 102 to 1591 days). During the follow-up period, 207 patients died, and 360 patients were readmitted due to CHF. No patient was lost to follow-up during the study.

Table 2 Baseline characteristics of the patients according to quartile levels of serum RBP4

Variables	1st quartile	2nd quartile	3rd quartile	4th quartile	P value
Age (years)	67 (66–73)	68 (67–74)	68 (66–76)	69 (67–75)	0.351
Male, n (%)	158 (67.8)	150 (64.1)	154 (66.1)	149 (63.7)	0.770
BMI (kg/m ²)	24.61 (22.86–26.64)	24.80 (22.47–26.71)	24.67 (22.52–26.83)	24.67 (22.19–26.54)	0.742
Smoking, n (%)	106 (45.5)	97 (41.5)	107 (45.9)	101 (43.2)	0.743
Hypertension, n (%)	85 (36.5)	106 (45.3)	101 (43.3)	100 (42.7)	0.242
Diabetes, n (%)	95 (40.8)	88 (37.6)	103 (44.2)	116 (49.6)	0.057
Ischaemic aetiology, n (%)	103 (44.2)	99 (42.3)	105 (45.1)	114 (48.7)	0.562
NYHA class					
I	50 (21.5)	49 (20.9)	48 (20.6)	37 (15.8)	0.386
II	73 (31.3)	69 (29.5)	79 (33.9)	65 (27.8)	0.518
III	74 (31.8)	75 (32.1)	74 (31.8)	87 (37.2)	0.523
IV	38 (16.3)	39 (16.7)	31 (13.3)	46 (19.7)	0.330
LVEF (%)	44 (37–52)	40 (34–53)	40 (34–51)	38 (34–46)	<0.001
NT-proBNP (pg/mL)	1349.17 (935.12–1608.91)	1507.83 (1265.88–1932.40)	1682.12 (1336.70–3297.59)	2634.96 (1571.93–4949.26)	<0.001
eGFR (mL/min/1.73 m ²)	78 (66–88)	73 (66–89)	72 (56–78)	68 (54–79)	<0.001
TC (mmol/L)	4.61 (3.97–5.11)	4.59 (3.94–5.08)	4.57 (3.89–5.15)	4.60 (3.97–5.32)	0.767
TG (mmol/L)	1.51 (1.13–2.02)	1.50 (1.04–1.97)	1.46 (1.06–2.23)	1.52 (1.06–2.19)	0.616
HDL-C (mmol/L)	1.07 (0.92–1.20)	1.08 (0.90–1.23)	1.07 (0.92–1.27)	1.07 (0.92–1.25)	0.914
LDL-C (mmol/L)	2.73 (2.31–3.32)	2.63 (2.18–3.15)	2.67 (2.29–3.28)	2.71 (2.26–3.30)	0.442
Medical treatment					
Diuretics, n (%)	227 (97.4)	229 (97.9)	222 (95.3)	218 (93.2)	0.038
Spironolactone, n (%)	182 (78.1)	192 (82.1)	197 (84.5)	141 (60.3)	<0.001
ACEI/ARB, n (%)	211 (90.6)	206 (88.0)	206 (88.4)	189 (80.8)	0.011
Beta-blocker, n (%)	128 (54.9)	145 (62.0)	134 (57.5)	90 (38.5)	<0.001

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor antagonist; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; RBP4, retinol-binding protein 4; TC, total cholesterol; TG, triglyceride.

Data are presented as median with interquartile range or number with percentage in parentheses.

Correlation between RBP4 and cardiac function

Serum RBP4 levels were increased as the NYHA class increased (Figure 1A). Serum RBP4 levels were 43.16 ± 8.44 , 43.96 ± 9.97 , 48.73 ± 13.39 , and 51.60 ± 15.51 $\mu\text{g/mL}$ in patients with NYHA Classes I–IV, respectively ($P < 0.001$). Serum RBP4 levels were also increased as LVEF decreased, with 43.70 ± 9.15 $\mu\text{g/mL}$ in HF with preserved ejection fraction (LVEF $\geq 50\%$), 45.32 ± 11.19 $\mu\text{g/mL}$ in HF with mid-range ejection fraction (LVEF 40–49%), and 48.73 ± 13.39 $\mu\text{g/mL}$ in HF with reduced ejection fraction (LVEF $< 40\%$) (Figure 1B). Moreover, serum RBP4 levels were negatively correlated with LVEF ($r = -0.154$, $P < 0.001$, Figure 1C) and positively correlated with NT-proBNP levels ($r = 0.074$, $P = 0.023$, Figure 1D).

Primary endpoint

Univariate Cox regression analyses showed that age, ischaemic aetiology, NYHA class, LVEF, log NT-proBNP, eGFR, and

log RBP4 were predictors for the primary endpoint of MACE (Table 3). In multivariable Cox regression analyses, log RBP4 was still associated with 1.6 times higher risk of MACE (HR = 2.61, 95% CI = 1.19–5.70), besides age (HR = 1.02, 95% CI = 1.01–1.04), male (HR = 1.24, 95% CI = 1.04–1.49), LVEF (HR = 1.06, 95% CI = 1.03–1.09), log NT-proBNP (HR = 2.63, 95% CI = 1.47–4.69), and eGFR (HR = 1.01, 95% CI = 1.00–1.01) (Table 3). Similar results were obtained by using serum RBP4 as a ranked variable (4th quartile vs. 1st quartile: adjusted OR = 1.39, 95% CI = 1.25–1.55, $P < 0.01$) (Table 4).

We further divided the CHF patients into diabetes and non-diabetes subgroups. Serum RBP4 levels were higher in patients with diabetes than those without diabetes (45.62 ± 11.70 $\mu\text{g/mL}$ vs. 48.03 ± 13.13 $\mu\text{g/mL}$, $P = 0.003$). Multivariable Cox regression analyses indicated that LVEF (HR = 1.04, 95% CI = 1.02–1.08), log NT-proBNP (HR = 3.40, 95% CI = 1.38–8.36), and log RBP4 (HR = 2.97, 95% CI = 1.09–5.71) were independent predictors for MACE in patients with diabetes, while age (HR = 1.02, 95% CI = 1.01–1.03), male (HR = 1.26, 95% CI = 1.04–1.51), LVEF

Figure 1 Association of RBP4 with the severity of cardiac dysfunction in elderly patients with CHF. (A) Changes of serum RBP4 in patients with different NYHA functional class. (B) Changes of serum RBP4 in patients with different LVEF subgroups. (C) Correlation of serum RBP4 levels with LVEF. (D) Correlation of serum RBP4 levels with NT-proBNP. CHF, chronic heart failure; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; NT-proBNP, N terminal pro brain natriuretic peptide; RBP4, retinol-binding protein 4; HFpEF, HF with preserved ejection fraction; HFmrEF, HF with mid-range ejection fraction; HFrfEF, HF with reduced ejection fraction.

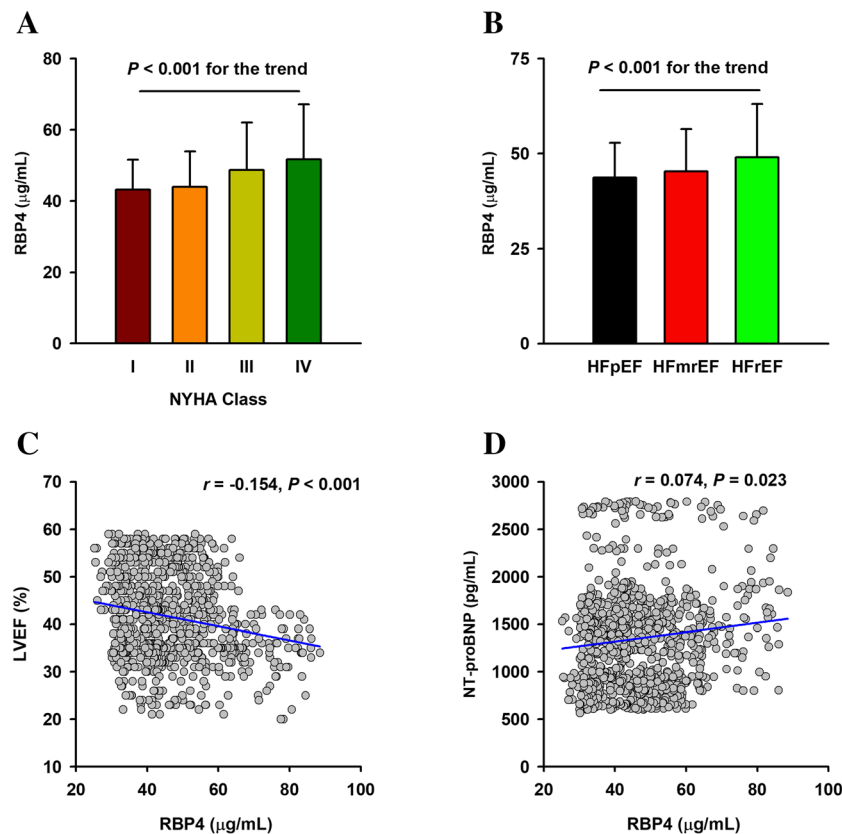


Table 3 Cox regression analyses for MACE

Variables	Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.06 (1.05–1.07)	<0.001	1.02 (1.01–1.04)	0.001
Male	1.10 (0.93–1.31)	0.277	1.24 (1.04–1.49)	0.017
BMI	1.00 (0.98–1.03)	0.918	1.00 (0.98–1.03)	0.989
Ischaemic aetiology	1.50 (1.27–1.77)	<0.001	0.96 (0.80–1.15)	0.634
Hypertension	0.98 (0.83–1.16)	0.801	1.04 (0.88–1.24)	0.625
Diabetes	0.96 (0.81–1.13)	0.585	0.88 (0.74–1.04)	0.129
Smoking	1.09 (0.92–1.28)	0.329	1.02 (0.86–1.21)	0.790
NYHA class	1.86 (1.70–2.04)	<0.001	1.06 (0.91–1.22)	0.454
LVEF	1.08 (1.05–1.11)	<0.001	1.06 (1.03–1.09)	<0.001
Log NT-proBNP	3.22 (1.83–7.54)	<0.001	2.63 (1.47–4.69)	0.001
eGFR	1.01 (1.01–1.02)	<0.001	1.01 (1.00–1.01)	0.042
Log RBP4	3.26 (1.92–7.27)	<0.001	2.61 (1.19–5.70)	0.016

BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; LVEF, left ventricular ejection fraction; MACE, major adverse cardiac event(s); NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; RBP4, retinol-binding protein 4.

Table 4 Associations of serum RBP4 with clinical outcomes in CHF patients

Model	1st quartile	2nd quartile	3rd quartile	4th quartile
MACEs				
Crude model	1	1.45 (1.10–1.90)*	1.47 (1.29–1.68)*	1.53 (1.40–1.66)*
Adjusted model	1	1.14 (0.85–1.55)	1.17 (0.99–1.38)	1.39 (1.25–1.55)*
Cardiovascular mortality				
Crude model	1	1.64 (1.41–1.91)*	1.73 (1.36–2.19)*	2.23 (1.38–3.60)*
Adjusted model	1	1.19 (0.89–1.62)	1.22 (0.91–1.63)	1.33 (1.09–1.61)*
Rehospitalization				
Crude model	1	1.25 (0.88–1.76)	1.35 (1.15–1.60)*	1.48 (1.34–1.64)*
Adjusted model	1	1.01 (0.69–1.47)	1.15 (0.95–1.41)	1.39 (1.22–1.59)*

CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiac event(s); RBP4, retinol-binding protein 4.

The adjusted model included age, sex, BMI, ischaemic aetiology, hypertension, diabetes, smoking, NYHA functional class, LVEF, NT-proBNP, eGFR, and medical treatments.

(HR = 1.06, 95% CI = 1.02–1.09), log NT-proBNP (HR = 2.69, 95% CI = 1.47–4.90), eGFR (HR = 1.01, 95% CI = 1.00–1.02), and log RBP4 (HR = 2.96, 95% CI = 1.31–6.67) were significant predictors for MACE in patients without diabetes (Supporting Information, Table S1). In addition, there was also no significant difference of serum RBP4 between men and women (47.21 ± 12.77 µg/mL vs. 46.37 ± 12.18 µg/mL, $P = 0.325$). Multivariable Cox regression analyses indicated that LVEF, log NT-proBNP, and log RBP4 were independent predictors for MACE both in male and female patients (Supporting Information, Table S2).

Secondary endpoints

Multivariable Cox regression analysis showed that log RBP4 was an independent predictor for cardiovascular mortality (HR = 2.24, 95% CI = 1.35–5.39) after adjustment for age, sex, BMI, ischaemic aetiology, hypertension, smoking, NYHA functional class, LVEF, log NT-proBNP, and eGFR. Moreover, our data indicated that log RBP4 was also

an independent predictor for CHF rehospitalization (HR = 2.54, 95% CI = 1.09–5.60) after adjustment for the aforementioned risk factors (Table 5). Similar results were obtained by using serum RBP4 as a ranked variable (4th quartile vs. 1st quartile: adjusted OR = 1.33, 95% CI = 1.09–1.61, $P < 0.01$ for cardiovascular mortality; adjusted OR = 1.39, 95% CI = 1.22–1.59, $P < 0.01$ for rehospitalization) (Table 4).

Kaplan–Meier survival analysis

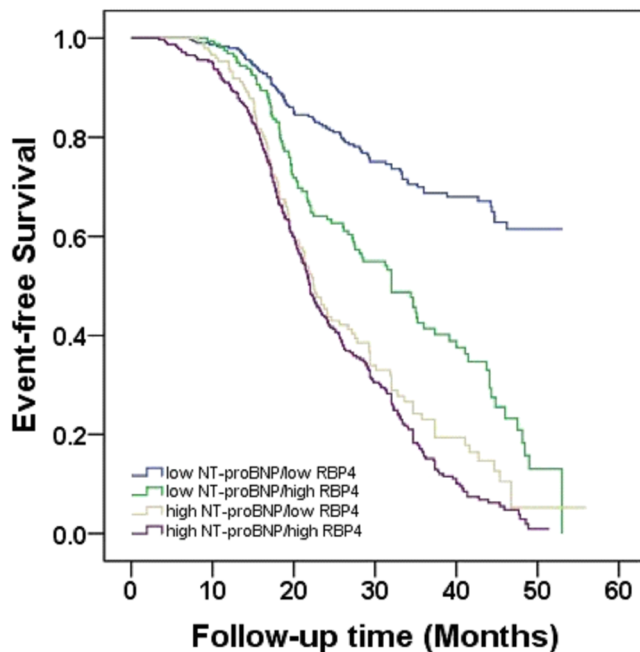
ROC curve analysis showed that the optimal cut-off value of RBP4 for the prediction of MACEs was 43.28 µg/mL, with a sensitivity of 65.8% and a specificity of 70.3% (area under the curve = 0.74, 95% CI = 0.71–0.77, $P < 0.001$). While for NT-proBNP, the optimal cut-off value was 1604.42 pg/mL, with a sensitivity of 67.7% and a specificity of 77.7% (area under the curve = 0.77, 95% CI = 0.74–0.80, $P < 0.001$). We further separated CHF patients into four groups according to the levels of NT-proBNP (1604.42

Table 5 Cox regression analyses for cardiovascular mortality and rehospitalization

Variables	Cardiovascular mortality		Rehospitalization	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.04 (1.02–1.07)	<0.001	1.03 (1.01–1.05)	0.002
Male	1.43 (1.05–1.94)	0.024	1.38 (1.08–1.76)	0.011
BMI	0.96 (0.92–1.01)	0.074	1.00 (0.96–1.04)	0.868
Ischaemic aetiology	2.86 (1.99–4.09)	<0.001	0.95 (0.74–1.21)	0.634
Hypertension	0.98 (0.73–1.30)	0.872	1.04 (0.83–1.31)	0.731
Smoking	1.19 (0.90–1.58)	0.227	0.99 (0.78–1.25)	0.920
NYHA class	1.47 (1.15–1.89)	0.003	0.97 (0.79–1.18)	0.454
LVEF	0.98 (0.95–1.01)	0.109	1.07 (1.05–1.09)	<0.001
Log NT-proBNP	3.17 (1.21–8.31)	0.019	2.63 (1.21–5.74)	0.015
eGFR	1.01 (0.99–1.02)	0.324	1.01 (1.00–1.02)	0.046
Log RBP4	2.24 (1.35–5.39)	0.021	2.54 (1.09–5.60)	0.035

BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; LVEF, left ventricular ejection fraction; MACE, major adverse cardiac event(s); NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; RBP4, retinol-binding protein 4.

Figure 2 Kaplan–Meier survival analysis. The event-free survival for MACE in elderly patients with CHF, stratified according to the cut-off values of RBP4 and NT-proBNP. CHF, chronic heart failure; MACE, major adverse cardiac event(s); NT-proBNP, N terminal pro brain natriuretic peptide; RBP4, retinol-binding protein 4.



pg/mL) and RBP4 (43.28 μ g/mL). Kaplan–Meier survival analysis showed that high RBP4 was a potential valuable predictive factor of MACE both in patients with low and high NT-proBNP levels (Figure 2). Patients with higher levels of RBP4 had a significantly higher incidence of MACE compared with those with lower levels of RBP4.

Discussion

To our knowledge, this is the first prospective cohort study that investigated the association of RBP4 with the prognosis of heart failure. The main finding of our study is that increased serum RBP4, a well-known adipokine with adverse effects on cardiovascular system, was correlated with the severity of cardiac dysfunction in elderly patients with CHF. Furthermore, elevated serum RBP4 levels were associated with worse outcome in elderly patients with CHF. Serum RBP4 appears to be a valuable additional prognostic marker for the risk stratification of elderly patients with CHF.

RBP4 is mainly secreted by liver and adipose tissue, and elevated circulating RBP4 levels have been linked to diabetes, obesity, and metabolic disorders.¹² In the present study, we also found that serum RBP4 levels were increased in patients with diabetes. Recently, growing evidence has suggested that RBP4 is actively involved in cardiovascular diseases, especially in the elderly population.⁶ Plasma RBP4 concentrations were increased in older patients with hypertension and correlated with the number of antihypertensive drugs.¹³ Another study of individuals aged over 70 years old showed that circulating RBP4 concentrations were associated with intima-media and plaque echogenicity in carotid arteries.¹⁴ Our previous study demonstrated that serum RBP4 was increased in patients with subclinical hypothyroidism and was associated with the presence and severity of coronary artery disease in patients with subclinical hypothyroidism.¹⁵ However, to date, data on the role of RBP4 in patients with CHF are scarce. We here showed that in the elderly patients with CHF, serum RBP4 levels were increased as the cardiac function decreased, which was quantified by the NYHA class and LVEF. Moreover, serum RBP4 levels were negatively correlated with LVEF and positively correlated with NT-proBNP in patients with CHF. The results are consistent with a previous study showing that

serum RBP4 levels were increased in patients with advanced heart failure and remarkably decreased in response to mechanical unloading and hemodynamic correction after the implantation of left ventricular assist device.⁷ However, another study of elderly subjects found no significant differences of plasma RBP4 levels between subjects with low levels (<125 ng/mL) and high levels (\geq 125 ng/mL) of NT-proBNP, as well as subjects with and without a history of hospitalization for heart failure.¹⁶ Moreover, no relationship was observed between the levels of RBP4 and NT-proBNP in their study.¹⁶ The discrepancies might partly attribute to the heterogeneity in study design. Our study population was restricted to elderly patients who were hospitalized for heart failure, while the study of Majerczyk *et al.*¹⁶ was a community-based study composed of both CHF patients and subjects with normal cardiac function. Another potential confounder might be renal function since RBP4 is mainly excreted by the kidney.^{17,18} Indeed, eGFR was lower in patients with high concentration of RBP4 in our study, indicating increased circulating RBP4 may serve as a biomarker for renal dysfunction in patients with CHF.

A series of adipokines have been reported as the predictors for unfavourable outcomes in patients with CHF.¹⁹ In the present cohort study, multivariate Cox regression analysis indicated that high level of RBP4 was an independent predictor for poor outcomes in the elderly patients with CHF even after adjustment for the well-known adverse prognostic factors for CHF. Similar results were found in the study of patients with ischaemic stroke, showing that elevated serum levels of RBP4 were associated with the severity and poor prognosis of acute ischaemic stroke.²⁰ However, another two studies of patients admitted in intensive care unit found that low serum RBP4 levels were related to increased mortality in patients with acute exacerbations of chronic obstructive pulmonary disease²¹ and underlying liver disease²², respectively. The decreased RBP4 levels might attribute to the malnutritional status in these critically ill patients, because circulating RBP4 concentration depends on vitamin A status.²³ It was reported that severe calorie restriction could reduce the circulating and adipose tissue messenger ribonucleic acid expression of RBP4.²⁴ Although sex hormones might affect the expression of RBP4 and its relationship with cardiovascular disease,²⁵ no significant difference of serum RBP4 levels was observed between male and female patients in our study. Moreover, our subgroup analysis found that log RBP4, age, LVEF, and log NT-proBNP were predictors of MACE both in male and female patients with CHF. Nevertheless, addition of RBP4 to the traditional risk factors may lead to an improvement in risk stratification for patients with CHF.

Previous experimental studies have revealed the prohypertrophic function of RBP4 in heart. Kraus *et al.*²⁶ found that angiotensin-II induced cardiac hypertrophy was reduced in RBP4 knockout mice. Moreover, recombinant RBP4 stimulation doubled the angiotensin-II induced hypertrophic

response in cardiomyocytes.²⁶ Consistently, our previous study showed that RBP4 enhanced protein synthesis, increased the cell size, and elevated expression of hypertrophic markers in cardiomyocytes.⁹ Interestingly, RBP4 also directly impairs glucose transporter-4 expression and insulin-stimulated glucose uptake in cardiomyocytes. There have been mounting evidence showing that insulin resistance and cardiac hypertrophy are not only associated but also they actually form a vicious cycle that leads to high mortalities in patients with CHF and diabetes.²⁷ Therefore, we speculated that RBP4 may be a key mediator that promotes the vicious cycle of insulin resistance and cardiac hypertrophy, which in turn causes the poor outcomes in patients with CHF. The mechanism underlying the effects of RBP4 in cardiac hypertrophy was related to RBP4-induced inflammation mediated by the activation of Toll-like receptor 4/myeloid differentiation primary response gene 88 pathway.⁹ However, additional in-depth investigation is required to elucidate the precise mechanism governing the pathological effect of RBP4 on the progression of CHF.

Study limitations

Firstly, as the present study was performed only in Chinese Han population, our findings need to be confirmed in other regions and ethnicities. Secondly, the patients in our study were all CHF patients but not acute heart failure patients. The prognostic role of RBP4 in patients with acute heart failure needs to be further delineated. Thirdly, we only measured baseline RBP4 levels and did not dynamically monitor the concentration of RBP4 during the follow-up period. Fourthly, further studies on animals and patients are needed to elucidate the exact role of RBP4 in the treatment of CHF. Finally, the role of residual confounding could not be entirely ruled out in observational studies.

Conclusions

In summary, our findings demonstrate for the first time that elevated serum RBP4 is correlated with worse outcome in elderly patients with CHF. Addition of RBP4 to the traditional risk factors may lead to an improvement in risk stratification for the elderly patients with CHF. However, long-term prospective cohort studies are still needed to confirm the prognostic value of RBP4 in heart failure.

Conflict of interest

None declared.

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Supporting information

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

Table S1. Cox regression analyses for MACE in patients with and without diabetes.

Table S2. Cox regression analyses for MACE in male and female patients.

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