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ORIGINAL ARTICLE

Prognostic value of cartilage intermediate layer protein 1 in chronic heart failure

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Abstract

Aims Emerging evidence suggests that cartilage intermediate layer protein 1 (CILP-1) is associated with myocardial remodelling. However, the prognostic value of circulating CILP-1 in patients with heart failure (HF) remains to be elucidated. This study aimed to investigate whether circulating CILP-1 can independently predict the outcome of chronic HF.

Methods and results This prospective cohort study included 210 patients with chronic HF and left ventricular ejection fraction <50% between September 2018 and December 2019. The primary endpoint was 1 year all-cause mortality. During the 1 year follow-up, 28 patients died. In multivariable Cox proportional hazards regression analysis, higher CILP-1 levels were independently associated with a higher risk of mortality after adjusting for potential confounding factors. In Kaplan–Meier analysis, patients with CILP-1 levels above the median had a significantly higher mortality rate than those with CILP-1 levels below the median (log-rank $P = 0.015$). In addition, CILP-1 significantly improved prognostic prediction over N-terminal pro-brain natriuretic peptide by an increase in net reclassification improvement ($P = 0.043$) and a trend towards an increase in integrated discrimination improvement ($P = 0.118$).

Conclusions Circulating CILP-1 is a novel independent prognostic predictor in chronic HF.

Keywords Heart failure; CILP-1; Prognosis; Biomarker

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Introduction

Heart failure (HF) is the leading cause of death, with an increasing prevalence worldwide.[1,2] Despite an improvement in survival rates with the development of novel therapies, HF mortality rate remains very high and larger than those of many types of cancer.[2,3] Identification of candidate prognostic biomarkers for HF can help guide individualized therapy.

Cartilage intermediate layer protein 1 (CILP-1) is an extracellular matrix (ECM) protein expressed predominantly in chondrocytes and involved in cartilage diseases, such as osteoarthritis and cartilage degeneration.[4–6] The CILP-1 gene codes for a 138 kD precursor protein that can be secreted in full length (F-CILP-1), or cleaved into a larger N-terminal (N-CILP-1) and a shorter C-terminal (C-CILP-1) fragment. All three protein variants have been reported to be functional; in particular, F-CILP-1 and N-CILP-1 can directly bind to transforming growth factor-β1 (TGF-β1) via the thrombospondin type 1 domain and inhibit its signalling pathway.[7,8] Of note, a growing number of studies suggest that CILP-1 is expressed in cardiac fibroblasts and exerts anti-fibrotic effects by interfering with TGF-β1 signalling.[7,9–11] Myocardial CILP-1 protein was found to be significantly up-regulated in animal models of left ventricular pressure overload,[8] acute myocardial infarction (AMI),[11] ischaemia/reperfusion injury,[12] and angiotensin II treatment.[9] Similar results have been observed in human myocardial tissues with AMI and HF.[11,13] In a single-cell transcriptomic analysis of cardiac fibrosis, fibroblast-CILP emerged as the most abundant fibroblast subpopulation.[9] In a mouse model of transverse aortic constriction, ventricular remodelling and dysfunction were significantly aggravated by CILP-1 knockdown but alleviated by CILP-1 delivery.[8]

As a novel secreted ECM protein, CILP-1 has the potential to be a sensitive marker for cardiac fibrosis. Park *et al.*[10]

found that although cardiac CILP-1 expression was significantly elevated, the level of F-CILP-1 in the serum of patients with HF was significantly lower than that in normal subjects. However, a recent study by Keranov *et al*.[14] found that circulating CILP-1 levels are significantly increased in patients with HF, especially with maladaptive right ventricular function. To date, no studies have evaluated the potential of CILP-1 as a prognostic biomarker for HF. Thus, this study aimed to investigate whether circulating CILP-1 can independently predict the outcome of chronic HF.

Methods

Study population

This prospective observational cohort study enrolled 210 chronic HF patients with left ventricular ejection fraction (LVEF) <50% from the First Affiliated Hospital of Guangxi Medical University between September 2018 and December 2019. Chronic HF was defined as New York Heart Association (NYHA) Class II or higher, based on the typical symptoms (e.g. breathlessness and fatigue), and/or signs (e.g. peripheral oedema, increased jugular venous pressure, and pulmonary crackles), and/or objective abnormality on echocardiography in line with the 2016 European Society of Cardiology guidelines.[15] The exclusion criteria were as follows: age <18 years, AMI, heart assist devices, clinical signs of infection, cancer, severe renal or hepatic function impairment, autoimmune disease, and psychosis. The primary endpoint of the study was 1 year all-cause mortality. Follow-up outcomes were obtained from hospital medical records or telephone interviews. In addition, we enrolled 35 healthy people from the physical examination centre of the hospital as a control group. The study protocol was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China, and fulfilled all principles of the Declaration of Helsinki. All the participants signed written informed consents.

Relevant definition

Body mass index was calculated as weight in kilograms divided by height in metres squared. Hypertension was defined as the presence of systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or a history of taking antihypertensive medications. Diabetes mellitus was defined according to the American Diabetes Association guidelines[16] or self-reported history of diabetes mellitus. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula.[17]

Cartilage intermediate layer protein 1 measurement

At the time of enrolment, blood samples were collected from all participants and drawn into dry tubes at room temperature for about half an hour. Blood samples were then centrifuged at 2600 *g* for 10 min, and the separated serum was stored at −80°C. Serum CILP-1 levels were measured by enzyme-linked immunosorbent assay kits (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Statistical analysis

Continuous variables were analysed using Student's *t*-test or Mann–Whitney *U* test as appropriate and described as mean ± standard deviation (SD) or median (inter-quartile range). Categorical variables were compared using χ^2 or Fisher's exact test and presented as counts (percentages). Spearman rank correlation and multivariate linear regression were performed to evaluate the association between CILP-1 levels and relevant clinical variables. The prognostic value of variables was determined using Cox proportional hazards regression. To assess the prognostic independence of CILP-1, we adjusted the univariate significant confounders and the variables that were independently correlated with CILP-1 levels. Model 1 was unadjusted. Model 2 was adjusted for N-terminal pro-brain natriuretic peptide (NT-proBNP). Model 3 was adjusted for NT-proBNP, diabetes, haemoglobin, uric acid, and eGFR. Furthermore, Kaplan–Meier analysis with log-rank testing was performed for 1 year survival analysis after dividing patients into two groups according to the median of CILP-1 concentrations. The added predictive value of CILP-1 over NT-proBNP for 1 year mortality was assessed by the area under the receiver operating characteristic (ROC) curve (AUC), continuous net reclassification improvement (NRI), and integrated discrimination improvement (IDI). Statistical tests were performed with the use of R statistical software Version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and MedCalc Version 18.11.3 (MedCalc, Mariakerke, Belgium). A two-tailed *P* value <0.05 was regarded statistically significant.

Results

Baseline characteristics

During the 1 year follow-up period, 28 patients (13.3%) experienced all-cause death. The main clinical characteristics of these patients are presented in *Table 1*. There was no significant difference between survivors and non-survivors in most data, such as age, sex, body mass index, blood pressure, heart

Table 1 Baseline characteristics

	All patients (n = 210)	Survivors (n = 182)	Non-survivors (n = 28)	P value
Age (years)	63.0 ± 11.3	62.9 ± 10.7	63.9 ± 15.0	0.681
Male gender, n (%)	183 (87%)	157 (86%)	26 (93%)	0.505
BMI (kg/m^2)	23.8 ± 3.8	23.9 ± 3.7	23.1 ± 3.9	0.293
Systolic blood pressure (mmHg)	124.5 ± 20.6	124.8 ± 20.6	122.5 ± 20.9	0.592
Diastolic blood pressure (mmHg)	75.9 ± 13.9	76.2 ± 13.8	74.0 ± 14.6	0.430
Heart rate at admission (b.p.m.)	85.1 ± 16.2	84.6 ± 16.6	88.6 ± 13.4	0.220
Current smoking, n (%)	73 (35%)	63 (35%)	10 (36%)	0.909
First diagnosis of HF >18 months, n (%)	66 (31%)	56 (31%)	10 (36%)	0.600
NYHA class, n (%)				0.107
II	111 (53%)	101 (56%)	10 (36%)	
III	57 (27%)	48 (26%)	9 (32%)	
IV	42 (20%)	33 (18%)	9 (32%)	
LVEF (%)	41.0 (35.0–45.0)	41.0 (35.0–45.3)	40.0 (31.3–45.0)	0.456
Ischaemic cause, n (%)	181 (86%)	157 (86%)	24 (86%)	1.000
Hypertension, n (%)	117 (56%)	104 (57%)	13 (46%)	0.288
Diabetes, n (%)	87 (41%)	75 (41%)	12 (43%)	0.869
Previous PCI/CABG, n (%)	70 (33%)	60 (33%)	10 (36%)	0.774
COPD, n (%)	5 (2%)	4 (2%)	1 (4%)	0.515
Atrial fibrillation/flutter, n (%)	18 (9%)	15 (8%)	3 (11%)	0.942
Cerebrovascular disease, n (%)	17 (8%)	15 (8%)	2 (7%)	1.000
Laboratory tests at admission				
WBC (× 10^9/L)	7.3 ± 2.3	7.3 ± 2.3	7.0 ± 2.3	0.503
Haemoglobin (g/L)	129.5 ± 22.3	131.5 ± 21.2	116.4 ± 25.4	0.001
LDL cholesterol (mmol/L)	2.7 ± 1.0	2.7 ± 1.0	2.6 ± 1.3	0.702
Triglyceride (mmol/L)	1.21 (0.90–1.80)	1.24 (0.92–1.80)	1.08 (0.72–1.80)	0.181
Uric acid (μmol/L)	476.6 ± 149.3	468.3 ± 149.4	531.0 ± 138.9	0.038
Creatinine (μmol/L)	94.5 (82.0–120.5)	93.0 (81.0–118.0)	114.0 (89.3–174.0)	0.016
eGFR (mL/min/1.73 m^2)	68.0 ± 23.8	69.3 ± 23.1	59.2 ± 27.0	0.036
NT-proBNP (pg/mL)	2675 (1148–6398)	2477 (1090–5815)	5566 (2387–18 315)	0.004
CILP-1 (ng/mL)	3.92 (2.62–6.12)	3.58 (2.55–5.60)	6.58 (2.95–8.60)	0.002
Medications at discharge, n (%)				
Beta-blocker	185 (88%)	162 (89%)	23 (82%)	0.465
ACEI/ARBs	153 (73%)	136 (75%)	17 (61%)	0.121
MRA	118 (56%)	101 (56%)	17 (61%)	0.604
Digoxin	56 (27%)	46 (25%)	10 (36%)	0.245
Diuretics	109 (52%)	91 (50%)	18 (64%)	0.159

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; CILP-1, cartilage intermediate layer protein 1; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SD, standard deviation; WBC, white blood cell.
Data are presented as mean ± SD (for normal distributions), or median (inter-quartile range, for skewed distributions), or number (percentage).

rate, smoking habit, time of first HF diagnosis, NYHA class, LVEF, medical history, white blood cell count, triglyceride level, low-density lipoprotein cholesterol level, and medication at discharge. However, non-survivors were characterized by higher levels of uric acid, serum creatinine, and NT-proBNP but lower levels of haemoglobin and eGFR compared with survivors. Notably, non-survivors had significantly higher CILP-1 levels than the survivors.

Furthermore, we measured CILP-1 levels from 35 healthy volunteers. The baseline information of the control group is presented in Supporting Information, *Table S1*. CILP-1 levels in the control group were significantly lower than both HF non-survivor group [3.11 (2.59–3.53) vs. 6.58 (2.95–8.60) ng/mL, $P < 0.001$] and HF survivor group [3.11 (2.59–3.53) vs. 3.58 (2.55–5.60) ng/mL, $P = 0.019$] (Supporting Information, *Figure S1*).

Association between baseline clinical variables and serum cartilage intermediate layer protein 1 levels

Spearman correlation analysis showed that serum CILP-1 levels were positively correlated with age, heart rate, uric acid levels, NYHA class, and NT-proBNP levels but negatively correlated with triglyceride levels and eGFR ($P < 0.05$). In addition, we found that serum CILP-1 levels were significantly higher in patients with diabetes, those with a history of percutaneous coronary intervention or coronary artery bypass graft, and those having HF duration for a long time ($P < 0.05$). To examine the independent determinants of CILP-1 variability, we performed a multivariate linear regression analysis with CILP-1 as a dependent variable (*Table 2*). NT-proBNP showed the strongest independent association

Table 2 Association between clinical variables and serum CILP-1 levels

	CILP-1		
	β coefficient	95% CI	*P*
Age	0.133	−0.009 to 0.276	0.067
Heart rate	0.040	−0.087 to 0.167	0.534
First diagnosis of HF >18 months	0.004	−0.282 to 0.290	0.976
NYHA class	0.140	−0.042 to 0.321	0.130
Diabetes	0.316	0.063–0.570	0.015
Previous PCI/CABG	0.196	−0.064 to 0.456	0.138
Haemoglobin	0.070	−0.059 to 0.200	0.283
Uric acid	0.123	−0.015 to 0.261	0.080
Triglycerides	−0.001	−0.127 to 0.125	0.988
eGFR	−0.002	−0.137 to 0.170	0.986
NT-proBNP	0.341	0.186–0.497	<0.001

CABG, coronary artery bypass grafting; CI, confidence interval; CILP-1, cartilage intermediate layer protein 1; eGFR, estimated glomerular filtration rate; HF, heart failure; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention.
Multivariate linear regression analysis with CILP-1 levels as a dependent variable. The β coefficient for the continuous variables is expressed as per 1-SD increase to allow comparison among effects.

Table 3 Cox proportional hazards regression analysis of CILP-1 for the prediction of mortality

	HR (95% CI)	*P* value
Model 1		
CILP-1, per 1 SD	1.61 (1.26–2.06)	<0.001
Model 2		
CILP-1, per 1 SD	1.37 (1.01–1.84)	0.040
NT-proBNP, per 1 SD	1.45 (1.09–1.94)	0.012
Model 3		
CILP-1, per 1 SD	1.52 (1.11–2.08)	0.009
NT-proBNP, per 1 SD	1.31 (0.89–1.91)	0.172
Diabetes	0.99 (0.46–2.13)	0.987
Haemoglobin, per 10 g/L	0.79 (0.67–0.94)	0.007
Uric acid, per 10 μmol/L	1.01 (0.98–1.03)	0.565
eGFR, per 10 mL/min/1.73 m^2	1.10 (0.90–1.34)	0.349

CI, confidence interval; CILP-1, cartilage intermediate layer protein 1; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; SD, standard deviation.
Model 1 was the unadjusted model; Model 2 was adjusted for NT-proBNP; and Model 3 was adjusted for NT-proBNP, diabetes, haemoglobin, uric acid, and eGFR.

with CILP-1 levels. In addition, diabetes was an independent positive determinant of CILP-1 variability. None of the other tested associations were significant (*Table 2*).

Cox regression analysis for 1 year mortality

In univariable Cox regression analysis, each 1-SD increase in serum CILP-1 levels was associated with a 1.61-fold ($P < 0.001$) increased risk of mortality (Model 1; *Table 3*). The risk remained strongly significant after adjustment for NT-proBNP in Model 2 [hazard ratio per 1-SD increase: 1.37; 95% confidence interval (CI), 1.01–1.84; $P = 0.004$] and after full adjustment in Model 3 (hazard ratio per 1-SD increase: 1.52; 95% CI, 1.11–2.08; $P = 0.009$; *Table 3*).

Kaplan–Meier analysis

In Kaplan–Meier analysis, patients with CILP-1 levels above the median had a significantly higher mortality rate than those with CILP-1 levels below the median (log-rank $P = 0.015$; *Figure 1*).

Incremental prognostic value of cartilage intermediate layer protein 1 over N-terminal pro-brain natriuretic peptide

Considering NT-proBNP as a classic prognostic biomarker of HF, we investigated whether CILP-1 can significantly improve the prediction of 1 year all-cause death over NT-proBNP. As shown in *Figure 2*, ROC curve analysis showed that both CILP-1 (AUC, 0.683; 95% CI, 0.615–0.745; $P = 0.004$) and NT-proBNP (AUC, 0.669; 95% CI, 0.601–0.732; $P = 0.005$) were good prognostic predictors, and there was no significant difference between the two ROC curves ($P = 0.808$). The addition of CILP-1 to NT-proBNP was not associated with a significant improvement in the AUC for prognostic prediction (AUC, 0.692 vs. AUC, 0.669; $P = 0.561$, *Table 4*). However,

Figure 1 Kaplan–Meier survival curves of 1 year all-cause mortality in patients grouped according to median cartilage intermediate layer protein 1 (CILP-1) level.

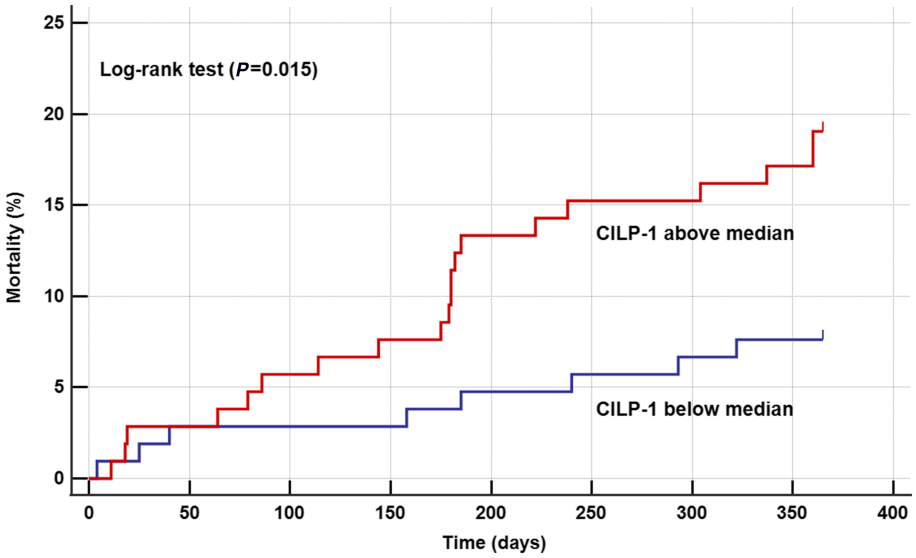
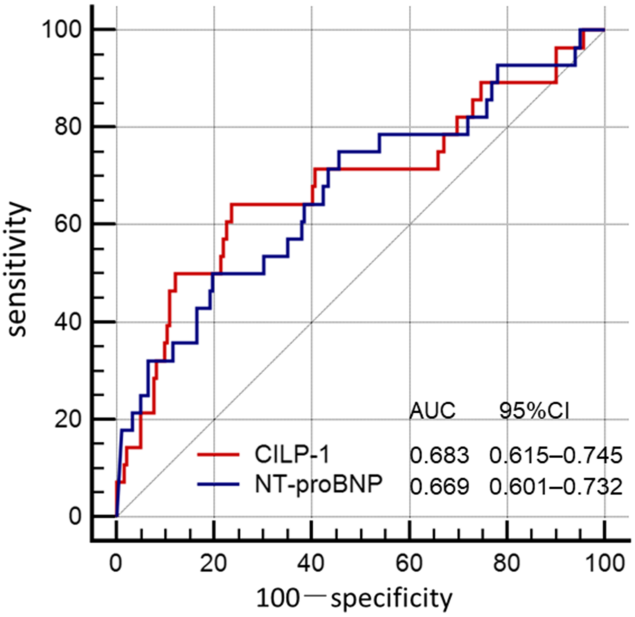


Figure 2 Receiver operating characteristic curve analysis of cartilage intermediate layer protein 1 (CILP-1) and N-terminal pro-brain natriuretic peptide (NT-proBNP) for predicting 1 year all-cause mortality. AUC, area under the receiver operating characteristic curve; CI, confidence interval.



CILP-1 significantly improved continuous NRI (NRI: 0.407, 95% CI: 0.013–0.800; *P* = 0.043) and tended to improve IDI (IDI: 0.030, 95% CI: −0.008 to 0.068; *P* = 0.118) over NT-proBNP (*Table 4*).

Discussion

The present study, for the first time, found that circulating CILP-1 is an independent predictor of mortality in chronic

Table 4 Improvement of mortality prediction by CILP-1 over NT-proBNP

Predictors	AUC (95% CI)	*P* value	Continuous NRI (95% CI)	*P* value	IDI (95% CI)	*P* value
NT-proBNP	0.669 (0.601–0.732)	Reference	Reference	Reference	Reference	Reference
NT-proBNP + CILP-1	0.692 (0.625–0.754)	0.561	0.407 (0.013–0.800)	0.043	0.030 (−0.008 to 0.068)	0.118

AUC, area under the receiver operating characteristic curve; CI, confidence interval; CILP-1, cartilage intermediate layer protein 1; IDI, integrated discrimination improvement; NRI, net reclassification improvement; NT-proBNP, N-terminal pro-brain natriuretic peptide.

HF and can significantly improve prognostic prediction over NT-proBNP.

Transforming growth factor-β1 plays an important role in the pathogenesis of cardiac remodelling by activating pro-fibrotic signalling pathways that promote ECM synthesis and myofibroblast transdifferentiation.[18] As an antagonist of TGF-β1 signalling, cardiac CILP-1 can be rapidly induced by TGF-β1,[7,10,14] thus creating a negative feedback loop. However, opposing observations regarding the circulating CILP-1 expression have been reported. Keranov *et al*.[14] reported that serum CILP-1 levels are significantly higher in patients with HF than in healthy controls, which is in line with our results showing that serum CILP-1 levels are positively correlated with NT-proBNP. However, Park *et al*.[10] reported that circulating F-CILP-1 levels were significantly reduced in HF, despite an abundance of cardiac expression. They assumed that increased F-CILP-1 is sequestered to the ECM by binding to TGF-β, thereby reducing its circulating levels. We speculate that one possible explanation for the opposing results is the difference in the enzyme-linked immunosorbent assay antibody targets. The precursor F-CILP can be directly secreted or cleaved into two fragments (N-terminal and C-terminal fragments). Park *et al*. used an antibody that spans the cleavage site of F-CILP-1 to specifically measure F-CILP-1 levels, while our and Keranov's studies used enzyme-linked immunosorbent assay kits targeting the N-terminal region (hence detecting both the N-CILP-1 and F-CILP-1). In parallel with increased precursor synthesis during cardiac remodelling, the enzyme activity involved in cleavage of the precursor is also probably enhanced, leading to increased N-CILP-1 but reduced F-CILP-1 levels. F-CILP-1 has been shown to inhibit TGF-β1 signalling by direct binding, similar to N-fragment,[7] but its function and temporal changes in the context of HF remain not fully understood. Given the evidence earlier, we assume that N-CILP-1, rather than the mixture of F-CILP-1 and N-CILP-1, may probably be the better biomarker for predicting the HF outcome. Further studies that selectively measure the different CILP-1 fragments are required in the future.

N-terminal pro-brain natriuretic peptide, which reflects myocardial strain, is one of the most extensively studied and validated prognostic biomarkers in HF.[19] However, the relatively low specificity of NT-proBNP limits its role as a single prognostic marker, and a combination of multiple biomarkers is required to improve the predictive accuracy. Following natriuretic peptides, many other biomarkers reflecting different HF pathophysiological processes (inflammation, myocardial injury, fibrosis, and remodelling) have been widely investigated, and some of them (such as cardiac troponins, soluble suppression of tumourigenesis-2, and galectin 3) have been recommended for prognostic risk stratification.[20–23] As a product of negative feedback, such as natriuretic peptides, circulating CILP-1 is associated with myocardial fibrosis and might represent HF development. Our study revealed that CILP-1 is an independent prognostic predictor in chronic HF, with an AUC similar to that of NT-proBNP, and that a combination of CILP-1 and NT-proBNP could improve predictive accuracy over NT-proBNP alone (improved NRI = 0.407, *P* = 0.043; a trend towards improved IDI = 0.030, *P* = 0.118). If validated in future large cohort studies, this finding would be of great clinical significance.

Despite the anti-fibrotic effect, we found that elevated circulating CILP-1 levels are associated with increased risk of death in patients with chronic HF. We speculate that this may be related to two reasons: (i) there was a significant positive correlation between the elevation of CILP-1 and TGF-β (the factor that promotes fibrosis). Keranov *et al*.[14] reported that TGF-β1 treatment in cardiac fibroblasts induced a significant increase in CILP-1 transcript, and CILP-1 expression was significantly correlated with the pro-fibrotic mediators at 72 h. However, increased endogenous expression of cardiac CILP-1 might not be sufficient to inhibit the strong effect of TGF-β[24]; thus, circulating CILP-1 might only represent disease severity. (ii) Pulmonary hypertension is a common complication of left HF, which can lead to right ventricular dilation and decompensation under long-term high pressure. The presence of pulmonary hypertension and right-sided HF suggests greater HF severity.[25,26] Intriguingly, recent evidence suggests that CILP-1 RNA expression is more pronounced in mouse models of right ventricular pressure overload than in left ventricular pressure overload.[27] Likewise, patients with maladaptive right ventricle showed significantly higher values of serum CILP-1 than those with adaptive right ventricle, dilative cardiomyopathy, or left ventricular hypertrophy.[14] Given that right-sided HF with severe fibrosis is generally accompanied by a poor prognosis,[25,26] it may partly explain the association of higher circulating CILP-1 levels with worse outcomes.

The limitations of the study were as follows: (i) given the single-centre design and small sample size with only a 1 year follow-up, the generalizability and precision of the results should be carefully considered. (ii) Although we collected baseline data as comprehensively as possible, residual poten-

tial confounders, such as unmeasured biomarkers, could not be entirely ruled out. (iii) Because our study only enrolled patients with chronic HF and LVEF $< 50\%$, it remains to be further elucidated whether CILP-1 is predictive of outcome in subjects with LVEF $\geq 50\%$. (iv) The dynamic re-examination of CILP-1 concentration, which may better predict the outcome, was not conducted in our study. More data and verification are required in the future.

In conclusion, circulating CILP-1 is a novel independent prognostic predictor in chronic HF.

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.

Figure S1. Comparison of CILP-1 levels in controls, HF survivors, and HF non-survivors.

Table S1. *Patient's characteristics of chronic heart failure and controls.*

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