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 remodeling. 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-$\beta$1 (TGF-$\beta$1) via the thrombospondin type 1 domain and inhibit its signalling pathway.7,8 Of note, a growing number of studies suggest that CILP-1 is expressed in cardiac fibroblasts and exerts anti-fibrotic effects by interfering with TGF-$\beta$1 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

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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. 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. 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 \( \geq 140 \text{ mmHg} \), or diastolic blood pressure \( \geq 90 \text{ mmHg} \), or a history of taking antihypertensive medications. Diabetes mellitus was defined according to the American Diabetes Association guidelines or self-reported history of diabetes mellitus. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula.

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 rate, and relevant clinical variables. The prognostic value of CILP-1 levels 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 \( \rho \) value <0.05 was regarded statistically significant.

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 \(-80^\circ \text{C}\). 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 \( \pm \) standard deviation (SD) or median (inter-quartile range). Categorical variables were compared using \( \chi^2 \) 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 \( \rho \) value <0.05 was regarded statistically significant.
Laboratory tests at admission

| Age (years)          | All patients (n = 210) | Survivors (n = 182) | Non-survivors (n = 28) | P value |
|----------------------|------------------------|---------------------|------------------------|---------|
| BMI (kg/m²)          | 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          | 86.6 ± 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 (%)    |                        |                     |                        |         |
| II                   | 111 (53%)              | 101 (56%)           | 10 (36%)               | 1.017   |
| III                  | 57 (27%)               | 48 (26%)            | 9 (32%)                |         |
| IV                   | 42 (20%)               | 33 (18%)            | 9 (32%)                |         |
| LVEF (%)             | 41.0 (35.0–45.0)       | 41.0 (35.0–45.0)    | 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 (× 10⁹/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)      | 93.1 (81.0–118.0)   | 114.0 (90.2–174.0)     | 0.016   |
| eGFR (mL/min/1.73 m²) | 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).

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.
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,

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

| Variable                          | 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 m² | 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.
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
HF and can significantly improve prognostic prediction over NT-proBNP.

The pathogenesis of cardiac remodelling by activating pro-fibrotic signalling pathways that promote ECM synthesis and myofibroblast transdifferentiation. As an antagonist of TGF-β1 signalling, cardiac CILP-1 can be rapidly induced by TGF-β1, thus creating a negative feedback loop. However, opposing observations regarding the circulating CILP-1 expression have been reported. Keranov et al. 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. 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, 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. 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. 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. 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-β24; 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. 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. 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. Given that right-sided HF with severe fibrosis is generally accompanied by a poor prognosis, 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 ≥ 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.

References

1. Savarese G, Lund LH. Global public health burden of heart failure. Card Fail Rev 2017; 3: 7–11.
2. Tomasoni D, Adamo M, Anker MS, von Haehling S, Coats AJS, Metra M. Heart failure in the last year: progress and perspective. ESC Heart Fail 2020; 7: 3505–3530.
3. Mamas MA, Sperrin M, Watson MC, Coutts A, Wilde C, Burton C, Kadam UT, Kwok CS, Clark AB, Murchie P, Buchan I, Hannaford PC, Myint PK. Do patients have worse outcomes in heart failure than in cancer? A primary care-based cohort study with 10-year follow-up in Scotland. Eur J Heart Fail 2017; 19: 1095–1104.
4. Tsuruha J, Masukohongo K, Kato T, Sakata M, Nakamura H, Nishioka K. Implication of cartilage intermediate layer protein in cartilage destruction in subsets of patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 2001; 44: 838–845.
5. Seki S, Tsumaki N, Motomura H, Nogami M, Kawaguchi Y, Hori T, Suzuki K, Yahara Y, Higashimoto M, Oya T, Ikegawa S, Kimura T. Cartilage intermediate layer protein promotes lumbar disc degeneration. Biochem Biophys Res Commun 2014; 446: 876–881.
6. Seki S, Kawaguchi Y, Chiba K, Mikami Y, Kizawa H, Oya T, Mio F, Mori M, Miyamoto Y, Masuda I, Tsumoda T, Kamata M, Kubo T, Toyama Y, Kimura T, Nakamura Y, Ikegawa S. A functional SNP in CILP, encoding cartilage intermediate layer protein, is associated with susceptibility to lumbar disc disease. Nat Genet 2005; 37: 607–612.
7. Shindo K, Asakura M, Min K-D, Ito S, Fu HY, Yamazaki S, Takahashi A, Imazu M, Fukuda H, Nakajima Y, Asanuma H, Minamino T, Takahisa S, Minamino N, Mochizuki N, Kitakaze M. Cartilage intermediate layer protein 1 suppresses TGF-β signaling in cardiac fibroblasts. Int J Gerontol 2017; 11: 67–74.
8. Zhang CL, Zhao Q, Liang H, Qiao X, Wang JY, Wu D, Wu L, Li L. Cartilage intermediate layer protein-1 alleviates pressure overload-induced cardiac fibrosis via interfering TGF-β1 signaling. J Mol Cell Cardiol 2018; 116: 135–144.
9. McLellan MA, Skelly DA, Dona MSL, Squiers FT, Farrugia GE, Gaynor TL, Cohen CD, Pandey R, Diep H, Vinał A, Rosenthal NA, Pinto AR. High-resolution transcriptomic profiling of the heart during chronic stress reveals cellular drivers of cardiac fibrosis and hypertrophy. Circulation 2020; 142: 1448–1463.
10. Park S, Ranbarvaziri S, Zhao P, Arehali R. Cardiac fibrosis is associated with decreased circulating levels of full-length CILP in heart failure. JACC Basic Transl Sci 2020; 5: 432–443.
11. van Nieuwenhoven FA, Munte C, Op’t Veld RC, González A. Cartilage intermediate layer protein (CILP1): a novel mediator of cardiac extracellular matrix remodelling. Sci Rep 2017; 7: 16042.
12. Barallobre-Barreiro J, Didangelas A, Schoendube FA, Drozdov I, Yin X, Fernández-Caggiano M, Willeit P, Puntmann VO, Aldama-López G, Shah AM, Doménech N, Mayr M. Proteomics analysis of cardiac extracellular matrix remodeling in a porcine model of ischaemia/reperfusion injury. Circulation 2012; 125: 789–802.
13. Shao X, Zhang X, Yang L, Zhang R, Zhu R, Feng R. Integrated analysis of mRNA and microRNA expression profiles reveals differential transcriptome signature in ischaemic and dilated cardiomyopathy induced heart failure. Epigenetics 2020; 16: 1–16.
14. Keranov S, Dör O, Jafari L, Troidl C, Liebetrau C, Kriechbaum S, Keller T, Voss S, Bauer T, Lorenz J, Richter MJ, Tello K, Gall H, Ghofrani HA, Mayer E, Wiedenroth CB, Guth S, Löhrchner H, Poling J, Chelladurai P, Pullamsetti SS, Braun T, Seeger W, Hamm CW, Nef H. CILP1 as a biomarker for right ventricular maladaptation in pulmonary hypertension. Eur Respir J 2021; 57: 1901192.
15. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Piecke B, Riley JP, Rosano GCM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016; 37: 2129–2200.
16. 2. Classification and diagnosis of diabetes—standards of medical care in diabetes—2020. Diabetes Care 2020; 43: S14–S31.
17. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604–612.
18. Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF-β) signaling in cardiac

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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ESCHF2.13746: 354–355
19. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masoudi FA, McBride PE, Peterson PN, Stevenson LW, Westlake C. 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. *Circulation* 2017; 136: e137–e161.

20. Sarhene M, Wang Y, Wei J, Huang Y, Li M, Li L, Acheampong E, Zhengcan Z, Xiaoyan Q, Yunsheng X, Jingyuan M, Xiumei G, Guanwei F. Biomarkers in heart failure: the past, current and future. *Heart Fail Rev* 2019; 24: 867–903.

21. Emdin M, Aimo A, Vergaro G, Bayes-Genis A, Lupón J, Latini R, Meessen J, Anand IS, Cohn JN, Gravning J, Gulstadi L, Broch K, Ueland T, Nymo SH, Brunner-La Rocca HP, de Boer RA, Gaggin HK, Ripoli A, Passino C, Januzzi JL Jr. sST2 predicts outcome in chronic heart failure beyond NT-proBNP and high-sensitivity troponin T. *J Am Coll Cardiol* 2018; 72: 2309–2320.

22. Bayes-Genis A, de Antonio M, Galán A, Sanz H, Urrutia A, Cabanes R, Cano L, González B, Diez C, Pascual T, Elosúa R, Lupón J. Combined use of high-sensitivity ST2 and NTproBNP to improve the prediction of death in heart failure. *Eur J Heart Fail* 2012; 14: 32–38.

23. van der Velde AR, Gulstadi L, Ueland T, Aukrust P, Guo Y, Adourian A, Muntendam P, van Veldhuisen DJ, de Boer RA. Prognostic value of changes in galectin-3 levels over time in patients with heart failure: data from CORONA and COACH. *Circ Heart Fail* 2013; 6: 219–226.

24. Groß S, Thum T. TGF-β inhibitor CILP as a novel biomarker for cardiac fibrosis. *JACC Basic Transl Sci* 2020; 5: 444–446.

25. Rosenkranz S, Gibbs JS, Wachter R, De Marco T, Vonk-Noordegraaf A, Vachiéry JL. Left ventricular heart failure and pulmonary hypertension. *Eur Heart J* 2016; 37: 942–954.

26. Thenappan T, Gomberg-Maitland M. Epidemiology of pulmonary hypertension and right ventricular failure in left heart failure. *Curr Heart Fail Rep* 2014; 11: 428–435.

27. Kreymborg K, Uchida S, Gellert P, Schneider A, Boettger T, Voswinckel R, Wietelmann A, Szibor M, Weissmann N, Ghofrani AH, Schermuly R, Schranz D, Seeger W, Braun T. Identification of right heart-enriched genes in a murine model of chronic outflow tract obstruction. *J Mol Cell Cardiol* 2010; 49: 598–605.