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ORIGINAL RESEARCH ARTICLE

# Circulating markers of collagen types I, III, and IV in patients with dilated cardiomyopathy: relationships with myocardial collagen expression

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## Abstract

**Aims** Collagen-derived peptides such as collagen I C-terminal telopeptide (CITP) and procollagen III N-terminal propeptide (PIIINP) have been conventionally used as markers of cardiac fibrosis. Collagen IV 7S domain (P4NP 7S) has been recently reported to be correlated with haemodynamics in patients with acute heart failure. We investigated whether these markers reflect cardiac remodelling and myocardial collagen expression.

**Methods and results** In 80 patients with dilated cardiomyopathy, relationships of CITP, PIIINP, and P4NP 7S to clinical and echocardiographic variables were analysed. CITP and PIIINP were inversely correlated with estimated glomerular filtration rate ($r = -0.41$, $P < 0.001$ and $r = -0.32$, $P = 0.004$, respectively); P4NP 7S was positively correlated with B-type natriuretic peptide ($r = 0.32$, $P = 0.003$) and γ-glutamyltransferase ($r = 0.38$, $P < 0.001$). These correlations were significant even after adjustment by potential confounders, whereas all three collagen markers were not independently correlated with ejection fraction nor with left ventricular (LV) diastolic diameter. In 33 patients undergoing endomyocardial biopsy, myocardial collagen I and III mRNA expressions were correlated with LV end-diastolic volume index ($r = 0.42$, $P = 0.02$ and $r = 0.54$, $P = 0.002$, respectively), whereas myocardial collagen IV mRNA expression was not correlated with LV end-diastolic volume index nor with ejection fraction. Each collagen-derived peptide was not significantly correlated with the myocardial expression of their corresponding collagen mRNA.

**Conclusions** Our study shows that CITP, PIIINP, and P4NP 7S do not reflect myocardial collagen mRNA expression but presumably reflect extra-cardiac organ injury in heart failure.

**Keywords** 7S domain of collagen IV (P4NP 7S); Fibrosis; C-terminal telopeptide of collagen I (CITP); N-terminal propeptide of procollagen III (PIIINP); Organ injury; Heart failure

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## Introduction

Organ fibrosis is a key pathological feature in a wide spectrum of diseases.[1] Circulating breakdown peptides of (pro-)collagen derived from collagen turnover have been extensively studied as markers for organ fibrosis, and some of them have been established as disease markers in the clinical settings.[2,3] In the field of cardiac diseases, the most consistent results have been found for the markers of collagens I and III, the dominant forms of collagen in the cardiac extracellular matrix (ECM).[4] They have been conventionally used as surrogates of cardiac fibrosis in a number of studies on ischaemia heart disease, hypertensive heart disease, or chronic heart failure (HF).[5–9] In addition, we and others have recently reported that breakdown peptide of 7S domain of collagen type IV (P4NP 7S), another type of

collagen composing the basement membrane in the ECM, was correlated with haemodynamics and had potential prognostic utility in patients with acutely decompensated HF.[10,11]

While cardiac fibrosis is the most important pathological feature of cardiac remodelling, emerging evidences suggest that haemodynamic disturbance accompanying HF involves extra-cardiac organs such as the kidneys and the liver.[12] Injury to those organs may activate pro-fibrotic response, leading to the release of collagen peptides. Because type I, III, and IV collagens are expressed in both cardiac and extra-cardiac ECM, there remains ambiguousness whether circulating peptides derived from these collagens reflect the pro-fibrotic milieu within the cardiac ECM or in the extra-cardiac organs.[13–15]

Therefore, we sought to investigate the relationships of circulating biomarkers of collage I [collagen I C-terminal telopeptide (CITP)], collagen III [procollagen III N-terminal propeptide (PIIINP)], and collagen IV (P4NP 7S) to clinical parameters, echocardiographic variables, and left ventricular (LV) myocardial expression of their corresponding collagen in patients with dilated cardiomyopathy.

# Methods

## Study design and population

This study included 80 patients with dilated cardiomyopathy, who were hospitalized for HF symptoms. All patients had systolic dysfunction [LV ejection fraction (LVEF) $<$ 50%], no coronary artery disease, no primary valvular disease, and no evidence of myocarditis or infiltrative cardiomyopathy. Patients with renal dysfunction (creatinine $>$3 mg/dL), known active neoplasia, active hepatitis or liver cirrhosis, and overt inflammatory, metabolic, or bone disease were excluded. All patients simultaneously underwent blood tests and echocardiography. Thirty-three patients underwent LV endomyocardial biopsies to determine the pathogenesis of new onset HF. In those patients, correlations of collagen expression levels in the biopsy specimens and collagen marker levels in the paired blood samples were analysed. This study was approved by the Ethics Committee of Osaka Red Cross Hospital. All study procedures followed the ethical principles of the Declaration of Helsinki; all patients provided written informed consent.

## Laboratory tests and assays of collagen markers

Blood samples were collected at stable condition after a median of 8 days of hospitalization and centrifuged for collection of serum and plasma. For those patients who required intravenous decongestive therapy, blood samples were collected after achieving acute-phase stabilization. Laboratory assays included renal and liver function tests (LFTs) and B-type natriuretic peptide (BNP). Serum samples for assays of collagen markers were refrigerated and transferred to a central laboratory where they were stored at $<-20$°C until used. Commercially available radioimmunoassay kits were used for CITP (Orion Diagnostica, Espoo, Finland), PIIINP (CIS Bio International, Codolet, France), and P4NP 7S (Sceti Medical Labo, Tokyo, Japan) assays. The lower limits of detection were 0.5 ng/mL for CITP, 0.2 U/mL for PIIINP, and 1.25 ng/mL for P4NP 7S. The coefficient of variation was $<$5%. Five patients were missing CITP data, and two were missing PIIINP data because of insufficient serum samples.

## Echocardiography

Echocardiography was routinely performed by experienced operator following American Society of Echocardiography guidelines.[16] Left atrial diameter and LV dimensions, mitral flow velocities, tricuspid regurgitant velocities, estimated pulmonary artery systolic pressure (ePA), tissue Doppler velocities, inferior vena cava (IVC) diameter, and LVEF were calculated.

## Catheterization and left ventricular endomyocardial biopsies

Selective coronary angiography and left ventriculography were performed via the femoral artery to determine LVEF, LV end-diastolic volume index (LVEDVI), and LV end-systolic volume index (LVESVI). Thereafter, LV endomyocardial biopsies were performed, using myocardial biopsy forceps (Technowood, Tokyo, Japan) after retrograde passage of the aortic valve. Tissues were immediately frozen at $-80$°C until RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) assay. Total RNA was isolated and purified using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized from 1 μg of total RNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) following the manufacturer's instructions. qRT-PCR with gene-specific primers was performed using an SYBR Green PCR master mix (Thermo Fisher Scientific, Waltham, MA, USA), and the products were analysed using a thermal cycler (ABI Prism 7900HT sequence detection system). The amplification of β-actin transcripts was used to normalize cDNA level. The following gene-specific primers were used: BNP, sense-GAGGAAGATGGACCGGATCA; antisense-TGTGGAATCAGAAGCAGGTGTCT; Collagen type I, sense-GGCGGCCAGGGCTCCGAC; antisense-AATTCCTGGTCTGGGGCACC; Collagen type III α1, sense-GGGAACAACTTGATGGTGCT; antisense-CCTCCTTCAACAGCTTCCTG; Collagen type IV α1, sense-GAAGGGTGATCCAGGTGAGA; antisense-CACCCTTGTCACCTTTTGGT; β-actin, sense-AGGCACTCTTCCAGCCTTCC; antisense-GCACTG


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TGTTGGCGTACAGG. Collagen IV expression data were missing from five patients because of insufficient tissue. No procedural complications occurred in the study population.

### Statistical analysis

Continuous variables were expressed as means value ± standard deviation or median and interquartile range. Between-group differences in the values of continuous variables were assessed by nonparametric Mann–Whitney *U*-tests. Correlations were tested for significance by the Spearman correlation coefficient. Factors independently associated with each collagen marker were identified by univariate and multivariate linear regression analyses. Clinically relevant factors listed in *Table 3* were simultaneously incorporated into the multivariate analyses after log transformation of collagen markers. Probability values $<0.05$ were considered as statistically significant. All statistical analyses were performed with JMP 10.0.0 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism 6.05 (GraphPad Software, Inc., La Jolla, CA, USA).

## Results

### Patient characteristics

The study population included 64% men, and more than 30% of the patients had severe HF symptoms with New York Heart Association class III or IV. Beta-blocker were prescribed for most patients; angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs) and aldosterone antagonists were prescribed for about half the patients at the time of blood sampling (*Table 1*). The mean LVEF was 31% ± 9% and the median LV diastolic dimension was 62 (57–68) mm. Proportion of patients with reduced LVEF ($<40\%$) and mid-range LVEF (40–49%) were 84% and 16%, respectively. The mean estimated glomerular filtration rate (eGFR) was 60.3 ± 18.0 mL/min/1.73 $m^2$, and the median BNP was 289 (178–918) pg/mL. The median serum concentrations of CITP, PIIINP, and P4NP 7S were 6.8 ng/mL, 0.7 U/mL, and 5.8 ng/mL, respectively (*Table 1*).

**Table 1** Clinical characteristics

| | |
|---|---|
| Clinical characteristics | |
| Age, years | 65 ± 13 |
| Male, % | 51 (64) |
| Hypertension, % | 30 (38) |
| Diabetes mellitus, % | 21 (26) |
| Dyslipidemia, % | 19 (24) |
| Atrial flatter or fibrillation, % | 36 (45) |
| NYHA III or IV | 25 (31) |
| Medication | |
| Beta-blocker, % | 75 (94) |
| ACE-I/ARB, % | 38 (48) |
| Aldosterone antagonist, % | 38 (48) |
| Loop diuretics, % | 57 (71) |
| Calcium channel blocker, % | 18 (23) |
| Anticoagulants, % | 25 (31) |
| Aspirin, % | 14 (18) |
| Statin, % | 15 (19) |
| Echocardiography | |
| EF, % | 31 ± 9 |
| LVDd, mm | 62 (57–68) |
| LVDs, mm | 54 (44–59) |
| MR ≥2 | 61 (78) |
| LAD, mm | 45 ± 7 |
| E/e' ratio | 17 (13–21) |
| ePA, mmHg | 34 (26–49) |
| IVC, mm | 18 (14–21) |
| Laboratory values | |
| T-Bil, mg/dL | 0.7 (0.6–1) |
| AST, U/L | 24 (20–34) |
| ALT, U/L | 24 (13–37) |
| ALP, U/L | 227 (190–270) |
| γ-GTP, U/L | 44 (30–73) |
| Creatinine, mg/dL | 0.9 (0.8–1.1) |
| BUN, mg/dL | 17.8 (14.2–23.5) |
| eGFR, mL/min/1.73 $m^2$ | 60.3 ± 18.0 |
| HbA1c, % | 5.9 (5.7–6.4) |
| BNP, pg/mL | 289 (178–918) |
| CITP, ng/mL | 6.8 (4.6–10.4) |
| PIIINP, U/mL | 0.7 (0.6–0.8) |
| P4NP 7S, ng/mL | 5.8 (4.7–7.1) |

ACE-I, angiotensin-converting enzyme inhibitor; ALP, alkaline phosphatase; ALT, alanine transaminase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; CITP, C-terminal telopeptide of collagen type I; EF, ejection fraction; eGFR, estimated glomerular filtration rate; ePA, estimated pulmonary artery systolic pressure; HbA1c, haemoglobin A1c; IVC, inferior vena cava; LAD, left atrial diameter; LVDd, left ventricular dimension diastolic; LVDs, left ventricular dimension systolic; MR, mitral regurgitation; NYHA, New York Heart Association; P4NP 7S, 7S domain of the collagen type IV N-terminal propeptide; PIIINP, N-terminal propeptide of procollagen type III; T-Bil, total bilirubin; γ-GTP, γ-glutamyltransferase.
Values are numbers (%), means ± standard deviation, or medians (interquartile range).

### Relationships of collagen markers to laboratory and echocardiographic variables

Serum CITP was correlated with E/e' ratio, eGFR, and BNP; PIIINP was correlated with peak early diastolic transmitral flow velocity (E), ePA, and eGFR (*Table 2*). P4NP 7S was correlated with most of the echocardiographic parameters including left atrial and ventricular dimensions, E and E/e' ratio, ePA, and IVC diameter. P4NP 7S was also correlated with laboratory test values including BNP and LFTs, such as total bilirubin, aspartate aminotransferase, and γ-glutamyltransferase (γ-GTP) but not with eGFR (*Table 2*). None of the tested collagen markers were correlated with LVEF (*Table 2*). No significant differences in serum concentrations of CITP, PIIINP, and P4NP 7S were observed between patients with reduced LVEF and patients with mid-range LVEF ($P = 0.2$, $P = 0.5$, and $P = 0.1$, respectively), nor between patients receiving ACE-Is/ARBs and not receiving ACE-Is/ARBs ($P = 0.1$, $P = 0.8$, and $P = 0.7$, respectively). In the multiple linear regression model, independent correlations were found between eGFR and


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**Table 2** Correlation of collagen markers with clinical parameters

| | CITP | | PIIINP | | P4NP 7S | |
|---|---|---|---|---|---|---|
| Variables | *r* | *P* | *r* | *P* | *r* | *P* |
| Echocardiography | | | | | | |
| EF | −0.22 | 0.06 | 0.14 | 0.2 | −0.15 | 0.2 |
| LVDd | 0.14 | 0.2 | −0.03 | 0.8 | 0.25 | 0.02 |
| LVDs | 0.16 | 0.2 | −0.1 | 0.4 | 0.22 | 0.047 |
| LAD | 0.22 | 0.06 | 0.18 | 0.1 | 0.33 | 0.003 |
| E | 0.18 | 0.1 | 0.33 | 0.004 | 0.51 | <0.001 |
| E/e' ratio | 0.27 | 0.03 | 0.22 | 0.06 | 0.29 | 0.01 |
| ePA | 0.14 | 0.23 | 0.23 | 0.04 | 0.41 | <0.001 |
| IVC | 0.22 | 0.054 | 0.16 | 0.16 | 0.35 | 0.001 |
| Laboratory values | | | | | | |
| T-Bil | 0.04 | 0.7 | −0.09 | 0.5 | 0.26 | 0.02 |
| AST | −0.07 | 0.5 | −0.05 | 0.7 | 0.32 | 0.004 |
| ALT | −0.17 | 0.2 | −0.18 | 0.1 | 0.19 | 0.1 |
| ALP | 0.13 | 0.3 | 0.03 | 0.8 | 0.15 | 0.2 |
| γ-GTP | −0.03 | 0.8 | −0.14 | 0.3 | 0.38 | <0.001 |
| eGFR | −0.41 | <0.001 | −0.32 | 0.004 | −0.04 | 0.7 |
| HbA1c | 0.02 | 0.9 | −0.11 | 0.4 | 0.02 | 0.9 |
| BNP | 0.36 | 0.002 | 0.04 | 0.7 | 0.32 | 0.003 |

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BNP, B-type natriuretic peptide; CITP, C-terminal telopeptide of collagen type I; EF, ejection fraction; eGFR, estimated glomerular filtration rate; ePA, estimated pulmonary artery systolic pressure; HbA1c, haemoglobin A1c; IVC, inferior vena cava; LAD, left atrial diameter; LVDd, left ventricular dimension diastolic; LVDs, left ventricular dimension systolic; P4NP 7S, 7S domain of the collagen type IV N-terminal propeptide; PIIINP, N-terminal propeptide of procollagen type III; T-Bil, total bilirubin; γ-GTP, γ-glutamyltransferase.

CITP, between E/e', eGFR, and PIIINP, and between BNP, E/e', γ-GTP, and P4NP 7S (*Table 3*). Importantly, all three collagen markers were not independently correlated with LVEF nor with LV diastolic diameter.

### Relationships of serum collagen metabolites and cardiac collagen mRNA expression

Quantitative real-time polymerase chain reaction analysis revealed that the expression of BNP mRNA level in LV tissue was significantly correlated with LVEF, LVEDVI, LVESVI, and with circulating BNP, as expected (*Table 4* and *Figure 1*). Expression levels of collagen I and III mRNA in LV tissue were significantly correlated with LVEDVI and LVESVI. Collagen I mRNA expression was also significantly correlated with circulating BNP and P4NP 7S. Of note, serum CITP and PIIINP levels were not significantly correlated with collagen I and III mRNA expressions (*Table 4* and *Figure 1*). Collagen IV mRNA expression was not significantly correlated with LVEF, LVEDVI, and LVESVI. Furthermore, it was neither correlated with circulating BNP nor with any collagen markers including P4NP 7S (*Table 4* and *Figure 1*).

## Discussion

In the present study, we investigated the relationships of circulating biomarkers of collagens I, III, and IV to clinical parameters, echocardiographic variables, and myocardial expression of their corresponding collagen. The main findings were as follows: (i) CITP and PIIINP were inversely correlated with eGFR, whereas P4NP 7S was positively correlated with LFTs and BNP. (ii) PIIINP and P4NP 7S were independently correlated with E/e'. However, all three markers were not independently correlated with echocardiographic indicators of LV remodelling such as LVEF and LV diastolic dimension. (iii) Myocardial collagen I and III mRNA expressions were correlated with LVEDVI, whereas collagen IV mRNA expression was not correlated with LVEDVI nor with LVEF. (iv) CITP, PIIINP, and P4NP 7S were not significantly correlated with the myocardial expression of the corresponding collagen mRNAs.

Cardiac fibrosis is the central pathological feature of cardiac remodelling. Because cardiac ECM contains type I and III collagens as dominant components, their circulating breakdown peptides have been classically assumed as biomarkers of cardiac fibrosis.[4,6] A number of large epidemiological studies reported an association of collagen markers with cardiac function and long-term prognosis.[5,7,8,17,18] In the present study, however, both CITP and PIIINP were not correlated with parameters of LV remodelling such as LVEF or LV dimensions, nor with cardiac expression of collagen types I and III.

**Table 3** Univariate and multivariate analyses of correlates of CITP, PIIINP, and P4NP 7S

| | Univariate analysis | | | | | | Multivariate analysis | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CITP | | PIIINP | | P4NP 7S | | CITP | | PIIINP | | P4NP 7S | |
| Variables | *β* | *P* | *β* | *P* | *β* | *P* | *β* | *P* | *β* | *P* | *β* | *P* |
| Age | 0.1 | 0.2 | 0.2 | 0.2 | −0.02 | 0.8 | −0.07 | 0.6 | −0.09 | 0.5 | −0.1 | 0.3 |
| γ-GTP | −0.07 | 0.6 | 0.1 | −0.2 | 0.3 | 0.005 | 0.02 | 0.9 | −0.1 | 0.2 | 0.3 | 0.008 |
| eGFR | −0.4 | <0.001 | −0.4 | <0.001 | −0.09 | 0.4 | −0.4 | 0.004 | −0.3 | 0.008 | −0.01 | 0.9 |
| BNP | 0.4 | <0.001 | 0.2 | 0.2 | 0.4 | <0.001 | 0.2 | 0.1 | 0.04 | 0.8 | 0.4 | 0.003 |
| E/e' | 0.2 | 0.045 | 0.3 | 0.02 | 0.3 | 0.003 | 0.1 | 0.4 | 0.3 | 0.04 | 0.3 | 0.03 |
| EF < 40% | 0.1 | 0.2 | −0.1 | 0.4 | 0.1 | 0.3 | 0.06 | 0.6 | −0.2 | 0.2 | −0.09 | 0.4 |
| LVDd | 0.2 | 0.08 | −0.01 | 0.9 | 0.3 | 0.009 | −0.008 | 1 | −0.1 | 0.4 | 0.1 | 0.4 |

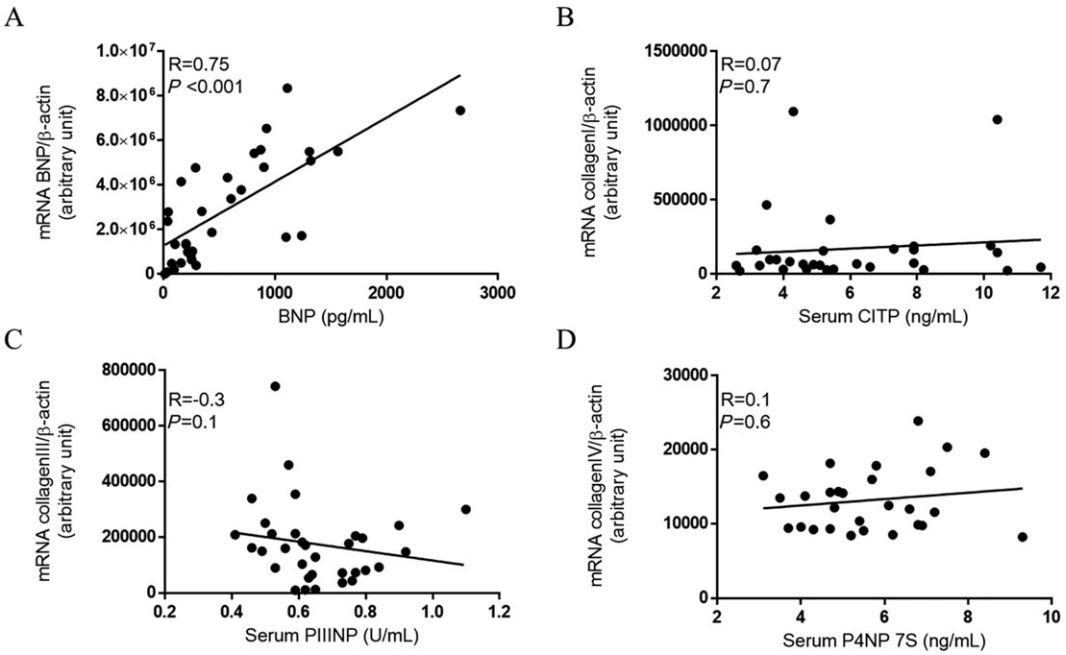
BNP, B-type natriuretic peptide; CITP, C-terminal telopeptide of collagen type I; EF, ejection fraction; eGFR, estimated glomerular filtration rate; LVDd, left ventricular dimension diastolic; P4NP 7S, 7S domain of the collagen type IV N-terminal propeptide; PIIINP, N-terminal propeptide of procollagen type III; *β*, standardized *β* coefficient; γ-GTP, γ-glutamyltransferase.

**Table 4** Relationships between cardiac collagen expression and LV functional parameters and serum collagen metabolites

| | mRNA BNP | | mRNA collagen I | | mRNA collagen III | | mRNA collagen IV | |
|---|---|---|---|---|---|---|---|---|
| Variables | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| EF | −0.45 | 0.01 | −0.14 | 0.4 | −0.3 | 0.1 | −0.15 | 0.5 |
| LVEDVI | 0.39 | 0.03 | 0.42 | 0.02 | 0.54 | 0.002 | 0.1 | 0.6 |
| LVESVI | 0.5 | 0.004 | 0.41 | 0.02 | 0.52 | 0.003 | 0.19 | 0.3 |
| BNP | 0.75 | <0.001 | 0.52 | 0.002 | 0.3 | 0.09 | 0.04 | 0.8 |
| CITP | 0.14 | 0.5 | 0.07 | 0.7 | −0.01 | 1 | 0.08 | 0.7 |
| PIIINP | −0.29 | 0.1 | −0.13 | 0.5 | −0.28 | 0.1 | 0.2 | 0.3 |
| P4NP 7S | 0.1 | 0.6 | 0.52 | 0.002 | 0.24 | 0.2 | 0.1 | 0.6 |

BNP, B-type natriuretic peptide; CITP, C-terminal telopeptide of collagen type I; EF, ejection fraction; LV, left ventricular; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; mRNA, messenger ribonucleic acid; P4NP 7S, 7S domain of the collagen type IV N-terminal propeptide; PIIINP, N-terminal propeptide of procollagen type III.

**Figure 1** Serum BNP, C-terminal telopeptide (CITP), procollagen III N-terminal propeptide (PIIINP), and 7S domain of collagen type IV (P4NP 7S) level and mRNA expression of the corresponding genes in the myocardium.




Because of limited volume of available tissue, evaluation of endomyocardial biopsy tissue is subject to the risk of sampling error. However, expression level of collagens I and III in the biopsy tissue was significantly correlated with increased LV volume, reflecting the well-known association between LV remodelling and progressive fibrosis. BNP expression in biopsy tissues was strongly correlated with blood BNP level, indicating that the expression levels in biopsy tissue did represent the pathological status of the whole myocardium. Thus, the lack of correlation of CITP and PIIINP with cardiac expression of their corresponding collagens implies that elevation of those markers does not reflect the increased expression of collagens I and III in the cardiac ECM. In consistent with our results, Kaye *et al.* recently reported that no net release of PIIINP was detected by simultaneous arterial and coronary sinus blood sampling from patients with advanced HF despite a significant correlation of arterial PIIINP with pulmonary wedge pressure and eGFR.[19] They also did not find any correlation of circulating CITP and haemodynamic parameters or transcardiac gradients of CITP.[20] These results suggest that elevation of circulating markers of collagens I and III in HF would be secondary to the decreased renal function and/or haemodynamic disturbance, rather than changes in the composition of cardiac ECM. Thus, caution should be paid for the interpretation of the results of clinical studies using these markers as surrogates of cardiac fibrosis.

7S domain of collagen type IV was significantly correlated with LFTs and BNP, as well as echocardiographic measurements including left atrial and ventricular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dimensions, ePA, IVC diameter, and E/e' ratio. Multiple regression analyses confirmed that P4NP 7S was related to both liver function and haemodynamics as indicated by independent correlations with γ-GTP, BNP, and E/e'. Type IV collagen is the major component of basement membrane spread in the ECM in multiple organs such as the heart and the liver. Previous animal studies reported that myocardial expression of collagen IV was increased in the pressure overload and infarction model,[13,21] indicating that the cardiac release is a potential source of circulating P4NP 7S in patients with HF. However, we found that expression of type IV collagen mRNA in LV biopsy tissues was not correlated with indicators of LV remodelling. Furthermore, serum P4NP 7S was not correlated with myocardial expression of type IV collagen, suggesting that circulating P4NP 7S does not reflect the pro-fibrotic response or remodelling of ECM within LV myocardium. On the other hand, there is clinicopathological evidence that P4NP 7S is a biomarker of liver fibrosis in primary liver diseases.[15,22] In HF, the liver is susceptible to haemodynamic stress-induced injury as reflected by elevation of LFTs in a significant proportion of patients.[23–25] Thus, significant correlation of P4NP 7S to LFTs, BNP, and echocardiographical variables in the present study implies that increased serum P4NP 7S in HF patients may be a result of accelerated turnover of collagen type IV in the liver likely due to systemic congestion. This speculation was also supported by the recent findings showing that P4NP 7S was significantly correlated with calculated liver fibrosis score or another liver fibrosis marker, hyaluronic acid.[10,26] Monitoring P4NP 7S in HF patients would provide information on the status of haemodynamic stress-induced organ injury and its pathological consequences. Notably, there are several reports showing that PIIINP is also elevated and reflects liver fibrosis in patients with primary liver disease.[27,28] However, we did not find any significant association between PIIINP and LFTs.

Despite the lack of significant correlation with myocardial collagen expression, collagen markers potentially help in understanding the pathophysiology of organ injury in HF and predicting cardiovascular outcome.[29] Further studies investigating how circulating concentrations of these collagen markers change during the disease course of HF and whether these markers have an additive prognostic utility on top of conventional risk markers among patients with HF are required.

## Limitations

The study has several limitations. First, not all markers derived from collagens I and III, such as carboxy-terminal propeptide of procollagen type I, were evaluated.[30] Second, serum biomarkers of collagen turnover may be influenced by co-morbidities.[4] To avoid this effect, patients with overt symptoms or abnormal laboratory tests suggesting the presence of these comorbidities were excluded. Third, because several different assays for the same collagen marker are available, care should be exercised when comparing collagen marker values across different studies. Finally, the small sample size limited the ability to assess the prognostic utility of collagen markers in this population.

## Conclusions

Circulating collagen markers do not accurately reflect myocardial collagen expression in patients with HF. Rather, changes of these markers seem to reflect extra-cardiac organ injury secondary to haemodynamic disturbance. Further studies are needed to investigate the precise mechanisms of elevation of collagen markers as well as their prognostic utility in patients with HF.

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## Conflict of interest

None declared.

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