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RESEARCH ARTICLE

# Significance of myocardial tenascin-C expression in left ventricular remodelling and long-term outcome in patients with dilated cardiomyopathy

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**Aim** Dilated cardiomyopathy (DCM) has a variety of causes, and no useful approach to predict left ventricular (LV) remodelling and long-term outcome has yet been established. Myocardial tenascin-C (TNC) is known to appear under pathological conditions, possibly to regulate cardiac remodelling. The aim of this study was to clarify the significance of myocardial TNC expression in LV remodelling and the long-term outcome in DCM.

**Methods and results** One hundred and twenty-three consecutive DCM patients who underwent endomyocardial biopsy for initial diagnosis were studied. Expression of TNC in biopsy sections was analysed immunohistochemically to quantify the ratio of the TNC-positive area to the whole myocardial tissue area (TNC area). Clinical parameters associated with TNC area were investigated. The patients were divided into two groups based on receiver operating characteristic analysis of TNC area to predict death: high TNC group with TNC area ≥2.3% (22 patients) and low TNC group with TNC area <2.3% (101 patients). High TNC was associated with diabetes mellitus. Comparing echocardiographic findings between before and 9 months after endomyocardial biopsy, the low TNC group was associated with decreased LV end-diastolic diameter and increased LV ejection fraction, whereas the high TNC group was not. Survival analysis revealed a worse outcome in the high TNC group than in the low TNC group ($P < 0.001$). Multivariable Cox regression analysis revealed that TNC area was independently associated with poor outcome (HR = 1.347, $P = 0.032$).

**Conclusions** Increased myocardial TNC expression was associated with worse LV remodeling and long-term outcome in DCM.

**Keywords** Dilated cardiomyopathy • Heart failure • Tenascin-C • Left ventricular remodelling • Prognosis

## Introduction

Dilated cardiomyopathy (DCM) is characterized by ventricular enlargement and impaired contractile function. The prognosis of DCM remains unfavourable, with a high incidence of sudden cardiac death and heart failure, and an estimated overall 1-year mortality rate between 10% and 30%, and some patients with DCM require heart transplantation.[1,2] Because of the widely diverse background characteristics of patients with DCM, the outcome of these patients is almost unpredictable, even with a variety of examinations, including blood tests, electrocardiography, chest X-ray, echocardiography, scintigraphy, and cardiac catheterization.

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Therefore, new prognostic indicators to stratify the future risk of DCM are strongly awaited.

Tenascin-C (TNC), a large hexametric glycoprotein, is known to appear in the extracellular matrix specifically following tissue injury or tumour formation where the particular organ is undergoing remodelling.[3] As regards the cardiac system, TNC is synthesized and expressed under pathological conditions in myocardial infarction, acute myocarditis, and DCM, suggesting a potential impact on the progression of cardiovascular diseases.[4,5] For example, TNC exacerbated heart failure by acceleration of left ventricular (LV) remodelling and an increase in fibrosis after myocardial infarction in a mouse model.[6] However, the effect of myocardial TNC expression with LV remodelling and long-term outcome in DCM has not yet been fully investigated. Similarly, the clinical background in which expression of TNC is increased in the myocardium has not been elucidated, although TNC is known to be synthesized by interstitial fibroblasts under inflammatory stimulation.[3]

The objective of this study was to investigate the association of myocardial TNC expression with LV remodelling and the long-term outcome. In addition, we examined the clinical factors of patients predisposed to increased tissue TNC expression in DCM.

# Methods

## Study population

Patients who were diagnosed with DCM in our hospital between 2005 and 2008 with impaired contractile function with left ventricular ejection fraction (LVEF) <40% and left ventricular end-diastolic diameter (LVEDD) >55 mm were screened for the study. All patients underwent clinical evaluation, physical examination, 12-lead electrocardiography, laboratory tests, echocardiography, and cardiac catheterization, including coronary angiography and right ventricular (RV) septal endomyocardial biopsy for initial diagnosis. All patients underwent RV endomyocardial biopsy according to the indications in the American Heart Association, American College of Cardiology, and European Society of Cardiology scientific statement, mainly to exclude myocarditis or secondary cardiomyopathy.[7] Endomyocardial biopsy was performed after obtaining written informed consent with information about the procedure and the risk of endomyocardial biopsy. Because medical therapy was optimized during hospitalization in most of the patients, information about the use of medication for heart failure such as $\beta$-blockers, angiotensin converting enzyme (ACE)-inhibitors or angiotensin receptor blockers (ARBs), and loop diuretics was collected separately on admission and at discharge. Patients with hypertrophic cardiomyopathy, ischaemic cardiomyopathy, valvular heart disease, relevant (LV) hypertrophy suggested to be caused by hypertensive heart disease, or secondary cardiomyopathy such as that caused by sarcoidosis or amyloidosis were excluded. Patients with a history of uncontrolled hypertension or LV assist device implantation were also excluded.

## Study protocol

Information about the patients' baseline characteristics, such as age, gender, body mass index, presenting symptoms, blood pressure, heart rate, previous comorbid conditions, and laboratory data (e.g. BNP, haemoglobin, serum creatinine), were collected from their medical records. Data on medication were collected both on admission and at discharge. Sections from stored paraffin-embedded RV septal tissue were stained with a specific antibody to TNC to evaluate TNC expression in the myocardium. Echocardiography was performed before and at 9 months after RV biopsy. Survival data were also collected in order to perform survival analysis. This study was performed according to the Helsinki Declaration and was approved by the ethics committee of our institution.

## Heart failure risk score

To evaluate heart failure severity, we used heart failure risk score accessible by the website http://www.heartfailurerisk.org.[8] This is the generalizable easily used integer risk score for mortality in patients with heart failure, including 13 independent predictors: age, LVEF, New York Heart Association (NYHA) class, serum creatinine, diabetes mellitus, $\beta$-blocker, systolic blood pressure, body mass index, time since diagnosis, current smoker, chronic obstructive pulmonary disease, male gender, and ACE inhibitor or ARB.

## Echocardiography

Echocardiographic parameters were measured according to the recommendations of the American Society of Echocardiography.[9] The LVEDD, left ventricular end-systolic diameter (LVESD), thickness of the interventricular septum and posterior ventricular wall, and left atrial diameter were obtained from M-mode or two-dimensional images of parasternal long axis views. Left ventricular end-diastolic diameter index (LVEDDI) was calculated as LVEDD divided by body surface area. LVEF was calculated by the Teichholz formula.[10] Mitral regurgitation was semiquantitatively graded from 1 to 4 considering the regurgitant area at colour Doppler imaging. Peak early (E), late (A) diastolic transmitral filling velocities, and deceleration time of E were measured, and E/A ratio was calculated. Restrictive filling pattern was defined as the E-wave deceleration time of <115 ms.[11]

## Cardiac catheterization

Patients underwent diagnostic cardiac catheterization. Coronary angiography was performed to exclude ischaemic heart disease, and right heart catheterization was performed to obtain haemodynamic data including cardiac output, pulmonary capillary wedge pressure (PCWP), and pulmonary artery pressure (PA). Endomyocardial biopsy was also performed, and three to five pieces of ventricular tissue were obtained from different locations in the septal wall of the right ventricle using disposable biopsy forceps (Technowood Co., Ltd., Tokyo, Japan). The specimens were immediately fixed with 10% buffered formalin, embedded in paraffin, serially sectioned and stained with haematoxylin and eosin staining. Masson's trichrome staining was also performed to detect fibrosis.

## Immunohistochemistry for tenascin-C

Immunohistochemical staining on formalin-fixed paraffin-embedded sections was performed using an autostainer Leica Bond-III (Leica Biosystems, Melbourne, Australia) according to the manufacturer's protocol. For antigen retrieval, slides were treated with Enzyme 1 using a Bond Enzyme Pretreatment Kit (AR9551;

**Table 1 Patient characteristics by tenascin-C (TNC) area**

| | Total, *n* = 123 | High TNC, *n* = 22 | Low TNC, *n* = 101 | *P*-value |
|---|---|---|---|---|
| Demographic characteristics | | | | |
| Age, years | 50.4 ± 14.5 | 45.9 ± 16.9 | 51.4 ± 13.9 | 0.186 |
| Male | 103 (84) | 20 (91) | 83 (82) | 0.317 |
| Body mass index, kg/m$^2$ | 23.8 ± 4.3 | 23.2 ± 4.8 | 23.9 ± 4.2 | 0.488 |
| Current smoker | 34 (28) | 4 (18) | 34 (34) | 0.156 |
| NYHA class III/VI | 41 (33) | 10 (46) | 31 (31) | 0.185 |
| Duration of heart failure, months | 21.6 ± 51.4 | 51.3 ± 92.2 | 15.1 ± 34.6 | 0.026 |
| Systolic blood pressure, mmHg | 116 ± 23 | 110 ± 22 | 117 ± 23 | 0.200 |
| Diastolic blood pressure, mmHg | 69 ± 12 | 67 ± 11 | 69 ± 12 | 0.706 |
| Heart rate, b.p.m. | 74 ± 15 | 75 ± 17 | 73 ± 14 | 0.639 |
| Hypertension | 49 (40) | 9 (41) | 40 (40) | 0.910 |
| Diabetes mellitus | 19 (15) | 8 (36) | 11 (11) | 0.003 |
| Dyslipidaemia | 63 (51) | 11 (50) | 52 (52) | 0.900 |
| Atrial fibrillation | 28 (23) | 8 (36) | 20 (20) | 0.095 |
| Left bundle branch block | 16 (13) | 4 (18) | 12 (12) | 0.439 |
| ICD/BiV-ICD | 8 (7) | 3 (14) | 5 (5) | 0.707 |
| Laboratory findings | | | | |
| Log BNP | 2.26 ± 0.59 | 2.59 ± 0.38 | 2.19 ± 0.61 | 0.003 |
| $HbA_{1c}$, % | 6.1 ± 0.9 | 6.2 ± 1.0 | 6.0 ± 0.9 | 0.455 |
| Haemoglobin, g/dl | 14.3 ± 1.9 | 14.2 ± 1.2 | 14.3 ± 2.0 | 0.905 |
| eGFR, mL/min.1.73 m$^2$ | 56.2 ± 20.9 | 55.2 ± 19.9 | 56.4 ± 21.2 | 0.448 |
| Serum sodium, mEq/L | 138 ± 4 | 139 ± 3 | 139 ± 5 | 0.577 |
| Medication on admission | | | | |
| β-blocker | 53 (43) | 12 (55) | 41 (41) | 0.233 |
| ACE inhibitor/ARB | 77 (63) | 16 (73) | 61 (60) | 0.281 |
| Loop diuretic | 81 (66) | 18 (82) | 63 (62) | 0.083 |
| Aldosterone inhibitor | 50 (41) | 13 (59) | 37 (37) | 0.057 |
| Sulphonylurea | 1(1) | 0 (0) | 1 (1) | 0.641 |
| Glinide | 2 (2) | 0 (0) | 2 (2) | 0.507 |
| Insulin | 2 (2) | 1 (5) | 1 (1) | 0.234 |
| Medication at discharge | | | | |
| β-blocker | 109 (89) | 20 (91) | 89 (88) | 0.710 |
| ACE inhibitor/ARB | 98 (80) | 21 (95) | 77 (76) | 0.043 |
| Loop diuretic | 78 (63) | 19 (86) | 59 (58) | 0.014 |
| Aldosterone inhibitor | 62 (50) | 15 (68) | 47 (47) | 0.067 |
| Sulphonylurea | 1 (1) | 0 (0) | 1 (1) | 0.641 |
| Glinide | 2 (2) | 1 (5) | 1 (1) | 0.234 |
| Insulin | 3 (2) | 1 (5) | 2 (2) | 0.481 |
| Echocardiographic findings | | | | |
| LVEDD, mm | 68.5 ± 8.4 | 70.2 ± 8.0 | 68.1 ± 8.5 | 0.179 |
| LVESD, mm | 59.4 ± 8.9 | 60.9 ± 9.3 | 59.1 ± 8.8 | 0.411 |
| LVEF, % | 25.2 ± 8.0 | 25.7 ± 8.0 | 25.1 ± 8.1 | 0.932 |
| LAD, mm | 46.1 ± 7.1 | 47.6 ± 6.7 | 45.8 ± 7.2 | 0.213 |
| RVD, mm | 29.9 ± 6.1 | 32.0 ± 6.9 | 29.4 ± 5.9 | 0.102 |
| Mitral regurgitation | 1.3 ± 0.9 | 1.3 ± 0.8 | 1.2 ± 0.9 | 0.579 |
| Restrictive filling pattern | 23 (19) | 8 (36) | 15 (15) | 0.020 |
| Haemodynamic data | | | | |
| Mean PCWP, mmHg | 12.1 ± 8.1 | 16.8 ± 8.6 | 11.1 ± 7.6 | 0.003 |
| Mean PA, mmHg | 20.0 ± 10.1 | 25.9 ± 10.1 | 18.7 ± 9.6 | <0.001 |
| Cardiac index, L/min.m$^2$ | 2.4 ± 0.6 | 2.2 ± 0.6 | 2.4 ± 0.6 | 0.171 |
| Heart failure risk score | 19.8 ± 6.6 | 22.0 ± 7.0 | 19.4 ± 6.4 | 0.116 |
| Collagen area, % | 14.4 ± 7.7 | 20.5 ± 10.5 | 13.1 ± 6.2 | 0.001 |

TNC, tenascin-C; NYHA, New York Heart Association; ICD, implantable cardioverter defibrillator; BiV-ICD, biventricular implantable cardioverter defibrillator; BNP, brain natriuretic peptide; $HbA_{1c}$, haemoglobin $A_{1c}$; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; RVD, right ventricular diameter; PCWP, pulmonary capillary wedge pressure; PA, pulmonary artery pressure.
Values are mean ± SD or *n* (%)

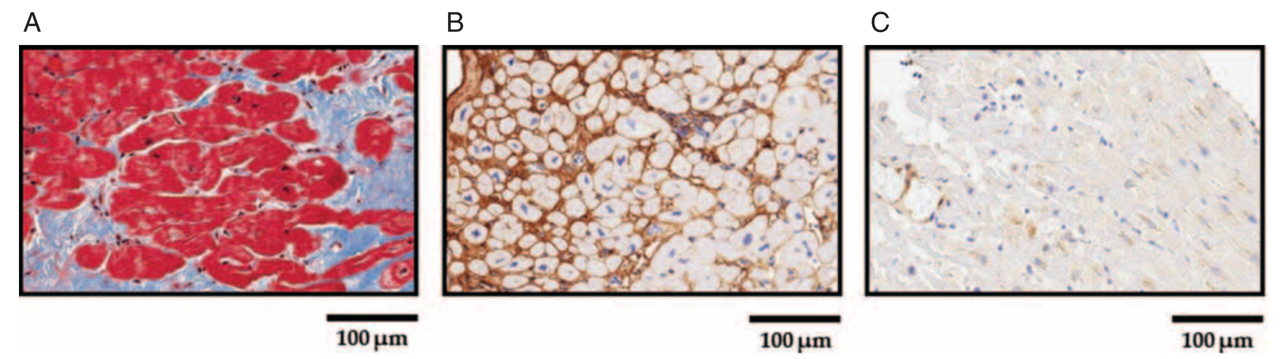


**Figure 1** Representative microscopic images of biopsy specimens. (A) Collagen area (blue-stained area) stained with Masson's trichrome was measured as 30.5%. (B) Tenascin-C (TNC)-positive area in immunohistochemical staining for TNC was measured as 9.9%, falling in the high TNC group. (C) TNC-positive area in immunohistochemical staining for TNC was measured as 0.5%, falling in the low TNC group.

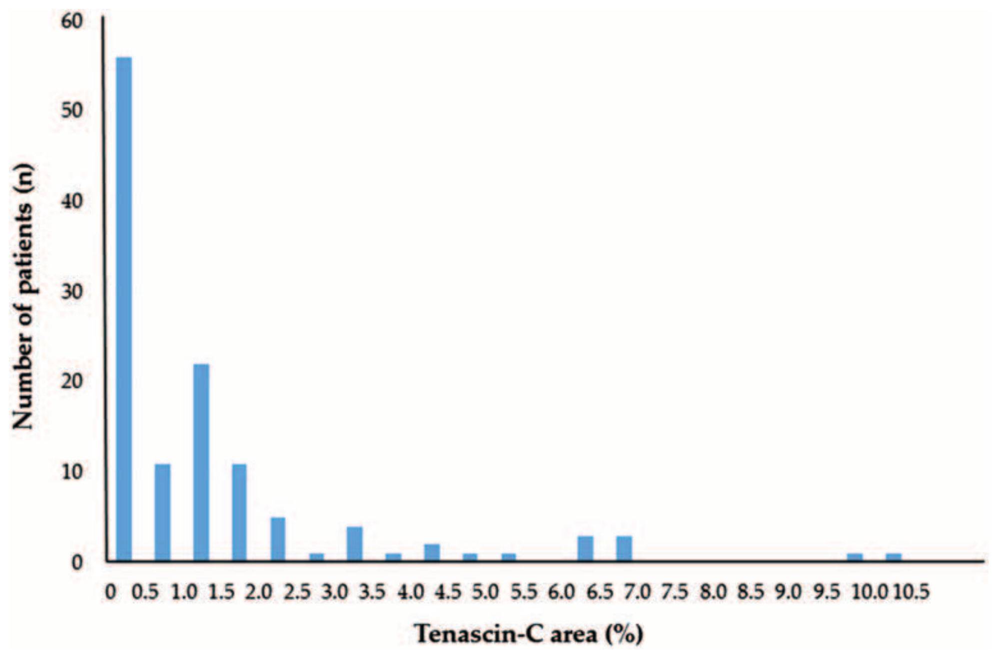


**Figure 2** Distribution of tenascin-C area. Mean tenascin-C area was 1.4 ± 2.0%, ranging from 0.1 to 10.4% with more than 80% of all patients showing a tenascin-C area <2.0%.

Leica Biosystems, Newcastle-upon-Tyne, UK) for 5 min at 37 °C. Slides were then incubated for 15 min at room temperature with a commercially available mouse monoclonal antibody for TNC (4F10TT, 1:1000 dilution; Immuno-Biological Laboratory Co., Ltd., Gunma, Japan), which recognizes epidermal growth factor (EGF)-like domain, constitutive sites of the TNC molecules.[12] Antibody detection and counterstaining with haematoxylin were performed using Bond Polymer Refine Detection Kit (DS9800; Leica Biosystems). Non-immunized mouse IgG (X0931, 1:1000 dilution; Dako, Glostrup, Denmark) was substituted as a negative control for the primary antibody against TNC to exclude possible false-positive responses from the secondary antibody or from non-specific binding of IgG.

## Analyses of myocardial collagen accumulation and tenascin-C expression

Myocardial collagen accumulation and TNC expression were semi-quantitatively measured in a blinded manner. Photomicrographs were taken in five different randomly-selected high-power fields in the low-magnification whole view of three to five pieces obtained by endomyocardial biopsy. Images were analysed with National Institutes of Health imaging software. The ratio of total blue-stained area without endocardium and blood vessels to whole myocardial area in Masson's trichrome-stained sections was calculated. The average value was taken to represent collagen accumulation and defined as the collagen area. Similarly, the TNC area was expressed as the average of the ratio of

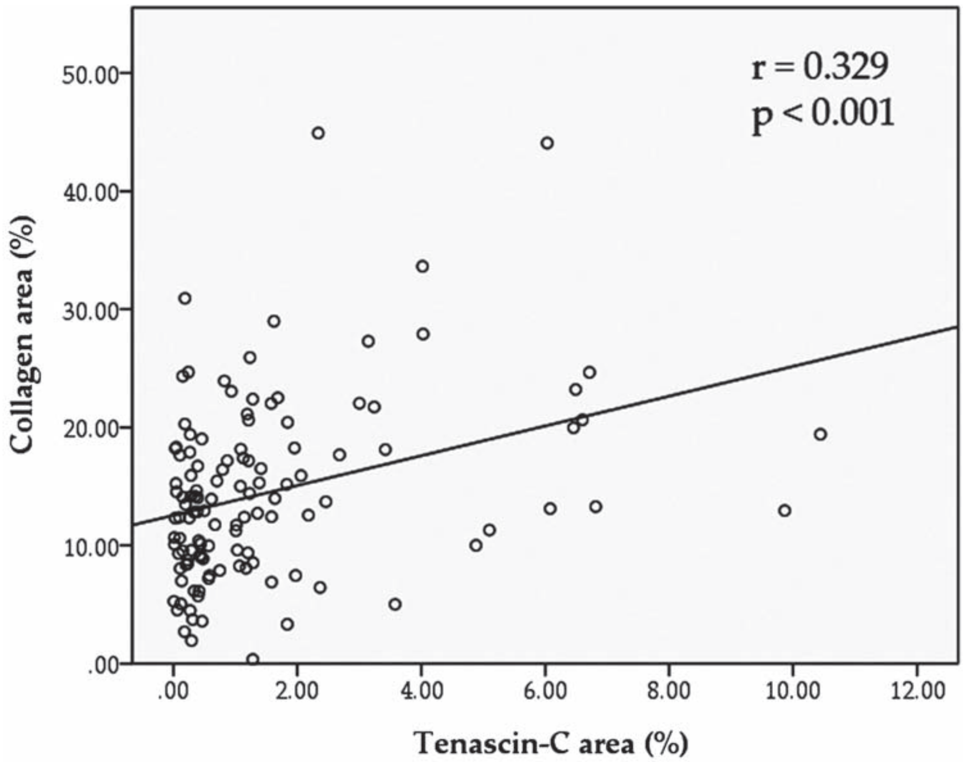


**Figure 3** Correlation between tenascin-C area and collagen area. Tenascin-C area was positively correlated with collagen area ($r = 0.329$, $P < 0.001$).

TNC-positive area to the whole myocardial area in immunohistochemically stained sections.

## Statistical analysis

Data were analysed using the Statistical Package for Social Sciences version 21.0 (SPSS Inc.; Chicago, IL, USA). All quantitative data were expressed as mean ± SD. The statistical significance of differences was analysed using unpaired Student's *t*-test for parametric continuous variables, the Mann–Whitney *U*-test for non-parametric continuous variables, and paired *t*-test to compare echocardiographic parameters at baseline and follow-up. Categorical variables were compared using Fisher's exact test. Pearson's correlation analysis was performed to estimate correlations between TNC area and fibrosis area. The receiver operating characteristic curve was constructed to determine the cut-off values. For survival analysis, the outcome of interest was death, and other patients were censored at the time of LV assist device implantation or heart transplantation, or at the day of last follow-up. Event-free survival curves were constructed using the Kaplan–Meier method, and the statistical significance of differences between curves was assessed using the log-rank test. Cox proportional hazard analysis was performed with 22 clinical variables that are generally recognized parameters influencing heart failure prognosis [age, body mass index, current smoker, NYHA class III or IV, duration of heart failure, heart rate, systolic blood pressure, diabetes mellitus, implantable cardioverter defibrillator (ICD) or biventricular ICD, logBNP, haemoglobin, estimated glomerular filtration rate, $\beta$-blocker at discharge, ACE inhibitor or ARB at discharge, aldosterone inhibitor at discharge, loop diuretic at discharge, LVEDDI, LVEF, mitral regurgitation, restrictive filling pattern, heart failure risk score, fibrosis area] and TNC area, and variables achieving $P < 0.05$ on univariable analysis were then tested in multivariable Cox analysis to determine which ones were significantly associated with death.[8] Univariable linear regression analysis for TNC area was performed using all variables in the baseline characteristics, and then multivariable linear regression analysis was performed using the variables achieving $P < 0.05$ on univariable linear regression analysis to determine the factors associated with TNC area. Values of $P < 0.05$ were considered statistically significant.

# Results

## Patient clinical characteristics

A total of 138 consecutive patients were finally diagnosed with DCM, and of these, 123 patients with complete data collection were included in the study. On admission, 43% and 63% of patients were receiving $\beta$-blockers and ACE inhibitors/ARBs respectively, but, at discharge, most of the patients were receiving these key medications for heart failure, suggesting that medications were optimized during hospitalization (*Table 1*). During the follow-up period of 66 ± 35 months, nine patients (7.3%) died and eight patients (6.5%) underwent LV assist device implantation. No patient underwent heart transplantation before LV assist device implantation.

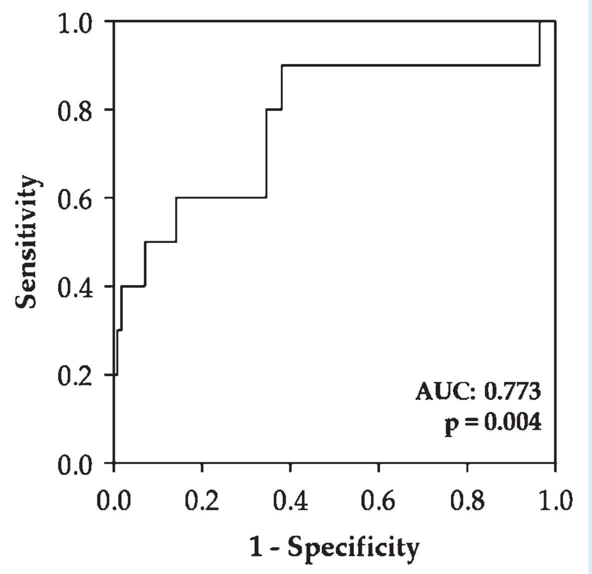


**Figure 4** Receiver operating characteristic analysis of tenascin-C area to predict death. The optimal cut-off value of tenascin-C area to predict death in patients with dilated cardiomyopathy was determined to be 2.3% based on the receiver operating characteristic curve [sensitivity = 0.600, specificity = 0.858; area under the curve (AUC) = 0.773, $P = 0.004$] in these study subjects.

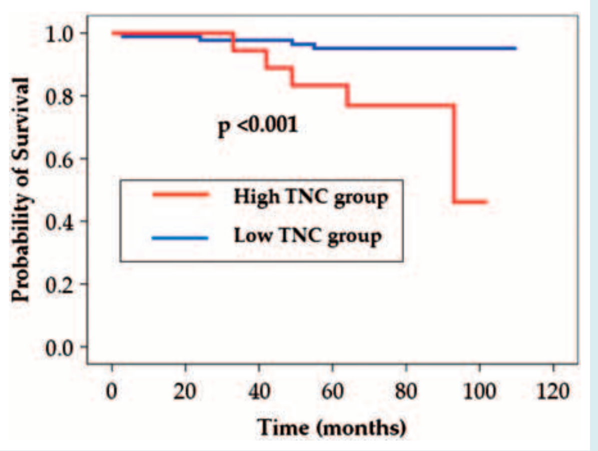


**Figure 5** Kaplan–Meier curves for all-cause mortality. Event-free survival was calculated for the high tenascin-C (TNC) group ($n = 22$) and low TNC group ($n = 101$) by Kaplan–Meier method. Survival rate was significantly decreased in the high TNC group compared with the low TNC group.

## Myocardial collagen area and myocardial tenascin-C expression

A representative image of a Masson's trichrome-stained section is shown in *Figure 1A* (collagen area 30.5%). Collagen area ranged from 0.4 to 44.9% (mean 14.4 ± 7.7%). Representative images of TNC-stained histological sections with high (*Figure 1B*, TNC area 9.9%) and low TNC (*Figure 1C*, TNC area 0.5%) expression are also shown. The distribution of TNC area is depicted in *Figure 2*. TNC area ranged from 0.1–10.4% (mean 1.4 ± 2.0%), with more than 80% of all patients having TNC area ≤2%. The TNC area was positively correlated with collagen area ($r = 0.329$, $p < 0.001$; *Figure 3*). Based on receiver operating characteristic analysis, the optimal cut-off value of TNC area to predict all-cause death in patients with DCM was determined to be 2.3% (sensitivity = 0.600, specificity = 0.858, area under the curve = 0.773, $P = 0.004$; *Figure 4*) in these study subjects. We divided the patients into two groups using this cut-off value; (i) high TNC group with TNC area ≥2.3% ($n = 22$, 18%), and (ii) low TNC group with TNC area <2.3% ($n = 101$, 82%).

## Patient characteristics and tenascin-C expression

Baseline characteristics were compared between the two groups (*Table 1*). Higher TNC expression was associated with longer duration of heart failure, higher incidence of diabetes mellitus and discharge, a loop diuretic at discharge and an ACE inhibitor or ARB at discharge. Mean PCWP, mean PA, plasma BNP level on admission, and collagen area in the high TNC group were higher than those in the low TNC group. However, baseline NYHA class, LV dimensions, LVEF, and heart failure risk score were not significantly different between the groups.

## Association of tenascin-C expression with outcome

Kaplan–Meier survival curves demonstrated that the high TNC group had a poorer outcome than the low TNC group ($P < 0.001$; *Figure 5*). Univariable and multivariable Cox regression analyses were performed to determine predictive factors for death. Systolic blood pressure, diabetes mellitus, ICD or biventricular ICD, log BNP, collagen area, and TNC area with a $P$-value <0.05 were selected by univariable analysis. Among these, multivariable analysis revealed that TNC area (hazard ratio = 1.347, $P = 0.032$) was an independent predictor of death (*Table 2*).

## Left ventricular remodelling

One patient died and two others had an LV assist device implanted before follow-up echocardiography. Follow-up echocardiographic data were unavailable in 27 patients. A total of 94 patients (15 patients in the high TNC group and 79 patients in low TNC group) underwent follow-up echocardiography performed 9 ± 4 months after the diagnosis of DCM. We found that LVEDD, LVESD and LAD were significantly decreased ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively), and LVEF and deceleration time were significantly increased ($P < 0.001$ and $P < 0.001$, respectively) at follow-up compared with baseline in the low TNC group, while these values did not differ between baseline and follow-up in the high TNC group (*Table 3*). To compare LV remodelling between the

**Table 2 Univariable and multivariable Cox regression analysis for death**

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| | HR (95%CI) | *P*-value | HR (95% CI) | *P*-value |
| Age | 0.990 (0.948–1.033) | 0.635 | – | – |
| Body mass index | 1.067 (0.937–1.215) | 0.329 | – | – |
| Current smoker | 0.401 (0.085–1.889) | 0.248 | – | – |
| NYHA class III/IV | 0.923 (0.238–3.577) | 0.908 | – | – |
| Duration of heart failure | 1.008 (1.000–1.016) | 0.063 | – | – |
| Heart rate | 0.987 (0.944–1.032) | 0.566 | – | – |
| Systolic blood pressure | 0.955 (0.918–0.993) | 0.020 | 0.981 (0.941–1.023) | 0.367 |
| Diabetes mellitus | 5.298 (1.534–18.302) | 0.008 | 4.323 (0.652–28.673) | 0.129 |
| ICD/BiV-ICD | 15.115 (2.912–78.457) | 0.001 | 7.801 (0.287–212.335) | 0.223 |
| Log BNP | 6.728 (1.725–26.242) | 0.006 | 6.661 (0.668–66.395) | 0.106 |
| Haemoglobin | 0.904 (0.649–1.259) | 0.904 | – | – |
| eGFR | 0.999 (0.967–1.032) | 0.941 | – | – |
| $\beta$-blocker at discharge | 0.446 (0.095–2.104) | 0.308 | – | – |
| ACE inhibitor/ARB at discharge | 0.558 (0.144–2.161) | 0.398 | – | – |
| Aldosterone inhibitor at discharge | 1.363 (0.384–4.838) | 0.631 | – | – |
| Loop diuretics at discharge | 1.783 (0.370–8.585) | 0.471 | – | – |
| LVEDDI | 0.986 (0.880–1.104) | 0.802 | – | – |
| LVEF | 1.028 (0.953–1.109) | 0.473 | – | – |
| Mitral regurgitation | 1.770 (0.841–3.723) | 0.132 | – | – |
| Restrictive filling pattern | 1.121 (0.233–5.400) | 0.886 | – | – |
| Heart failure risk score | 0.986 (0.908–1.103) | 0.986 | – | – |
| Collagen area | 1.089 (1.007–1.178) | 0.032 | 1.016 (0.894–1.154) | 0.808 |
| Tenascin-C area | 1.502 (1.243–1.814) | <0.001 | 1.347 (1.026–1.768) | 0.032 |

HR, hazard ratio; CI, confidence interval; BiV-ICD, biventricular implantable cardioverter defibrillator; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEDDI, left ventricular end-diastolic diameter index; LVEF, left ventricular ejection fraction.

**Table 3 Echocardiographic parameters at baseline and follow-up**

| | High TNC (*n* = 15) | | | Low TNC (*n* = 79) | | |
|---|---|---|---|---|---|---|
| | Baseline | Follow-up | *P*-value | Baseline | Follow-up | *P*-value |
| LVEDD, mm | 69.5 ± 8.6 | 64.7 ± 8.3 | 0.161 | 68.6 ± 9.2 | 53.4 ± 10.6 | <0.001 |
| LVESD, mm | 59.9 ± 9.6 | 54.4 ± 8.4 | 0.174 | 59.7 ± 9.5 | 46.6 ± 12.6 | <0.001 |
| IVST, mm | 8.8 ± 2.8 | 8.7 ± 2.7 | 0.928 | 8.7 ± 2.2 | 9.2 ± 2.1 | 0.094 |
| PWT, mm | 9.1 ± 2.2 | 8.8 ± 1.3 | 0.928 | 9.1 ± 2.1 | 9.2 ± 2.0 | 0.695 |
| LVEF, % | 26.6 ± 8.2 | 29.8 ± 8.2 | 0.285 | 24.8 ± 8.2 | 39.8 ± 13.4 | <0.001 |
| RVD, mm | 32.5 ± 7.3 | 31.2 ± 6.6 | 0.780 | 29.5 ± 5.8 | 28.6 ± 5.0 | 0.290 |
| E, cm/s | 82.7 ± 29.3 | 68.2 ± 22.2 | 0.183 | 70.1 ± 28.6 | 63.8 ± 22.9 | 0.095 |
| E/A | 2.1 ± 1.0 | 1.1 ± 0.7 | 0.229 | 1.1 ± 0.6 | 1.0 ± 0.6 | 0.840 |
| DcT, ms | 145 ± 70 | 177 ± 61 | 0.139 | 169 ± 56 | 207 ± 56 | <0.001 |
| LAD, mm | 49.3 ± 6.6 | 44.8 ± 11.5 | 0.134 | 46.4 ± 7.1 | 41.7 ± 7.9 | <0.001 |

TNC, tenascin-C; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVST, interventricular septum thickness; PWT, posterior left ventricular wall thickness; LVEF, left ventricular ejection fraction; RVD, right ventricular diameter; E, peak early diastolic transmitral filling velocity; E/A, peak early diastolic transmitral filling velocity/peak late transmitral filling velocity ratio; Dct, deceleration time; LAD, left atrial diameter.
Values are mean ± SD.

high and low TNC groups, we evaluated the changes in echocardiographic parameters from baseline to follow-up. Changes in LVEDD ($P = 0.041$) and LVESD ($P = 0.010$) were significantly smaller in the low TNC group, and change in LVEF ($P = 0.003$) was greater in the low TNC group compared with the high TNC group, suggesting that LV reverse remodelling was less prone to be induced during the follow-up period in the high TNC group (*Figure 6*).

## Determinants of tenascin-C area

Univariable and multivariable linear regression analysis were performed to determine the background characteristics associated with the high TNC area. All variables in the baseline patient characteristics were selected for univariable analysis. Among these variables, diabetes mellitus, ICD or biventricular ICD, mean

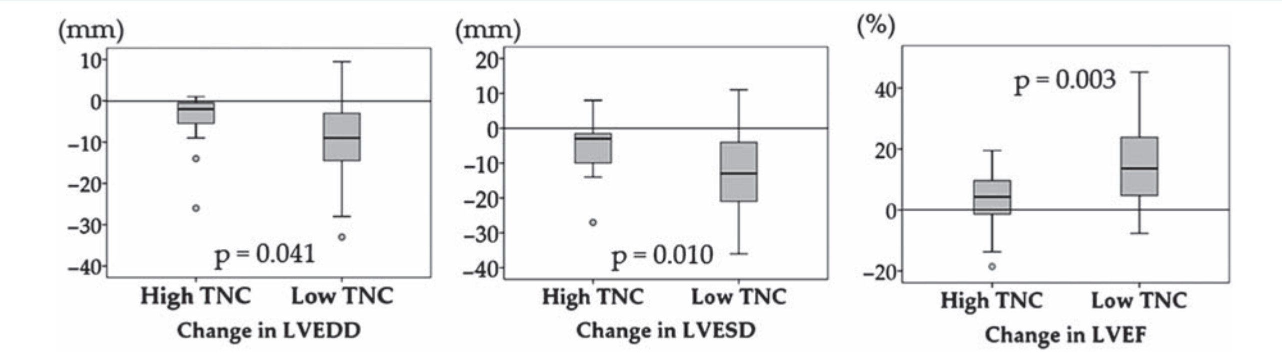


**Figure 6** Changes in left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), and left ventricular ejection fraction (LVEF) in high tenascin-C (TNC) group and low TNC group. The bottom and top of the box are the first and third quartiles, and the band inside the box is the median. The ends of the whiskers denote 1.5 interquartile range of the lower and upper quartiles. The circles represent outliers. Changes in LVEDD ($-9.2 \pm 8.6$ mm vs. $-4.9 \pm 7.2$ mm) and LVESD ($-13.0 \pm 10.4$ mm vs. $-5.5 \pm 8.5$ mm) were significantly smaller in the low TNC group compared with the high TNC group. The change in LVEF was significantly smaller in the high TNC group ($3.2 \pm 10.8$% vs. $15.0 \pm 12.6$%). These data suggest that the high TNC group was associated with lower occurrence of reverse remodelling in patients with dilated cardiomyopathy.

PCWP, and mean PA were shown to be independent determinants of high TNC area by multivariable analysis (*Table 4*).

# Discussion

In the present study, we found that myocardial TNC expression, immunohistochemically stained and analysed semiquantitatively in RV biopsy samples, was a useful predictor of LV remodelling and long-term outcome.

The outcome of DCM varies greatly among individual cases and is closely associated with the degree of LV remodelling and whether cardiac function is improved by conventional therapy, including $\beta$-blockers and ACE-inhibitors.[13,14] Left ventricular remodelling is affected by various kinds of neurohumoral and local factors such as the renin–angiotensin–aldosterone system, the adrenergic nervous system, increased oxidative stress, proinflammatory cytokines, and endothelin.[13–17] However, the precise mechanisms that cause LV remodelling are still unclear.

Ventricular extracellular matrix proteins, which maintain cardiac structure and architecture in combination with myocytes, play a central role in LV remodelling in DCM.[18] Recently, several studies have shown that TNC, one of the extracellular matrix component proteins, is expressed in association with several cardiovascular diseases.[19,20] This large glycoprotein (>300 kDa) is expressed only during the development of the embryonic heart and not in the normal adult heart.[21] However, when exposed to mechanical or ischaemic stress, it reappears in the heart. For example, TNC is expressed after myocardial infarction, in acute or chronic myocarditis, and in DCM in response to tissue injury and inflammation.[4,5] Previously, serum TNC level was reported to be related to disease progression and to have prognostic significance in DCM. Serum measurement of TNC level, because of its less invasive nature, could serve as a potential clinical biomarker reflecting TNC expression.[19,20,22] In the present study, by directly staining myocardial biopsy specimens, we demonstrated that high TNC expression was related to a worse outcome and lower occurrence of reverse remodelling in patients with DCM. As TNC is not exclusively synthesized in the heart, but also in organs such as the liver and lungs,[3] direct histological investigation of myocardial expression using a specific antibody appears to have great significance.

Tenascin-C has several functions that weaken cell adhesion, upregulate the expression and activity of matrix metalloproteinases, modulate inflammatory response, promote recruitment of myofibroblasts, and enhance fibrosis.[3] In an experimental myocardial infarction model using genetically altered mice, TNC deficiency ameliorated collagen accumulation in the infarct border area, resulting in improved post-myocardial infarction cardiac function.[6] Tenascin-C has the structural capability to bind and interact with other cells. This large extracellular matrix glycoprotein, consisting of EGF-like repeats, fibronectin III repeats, and a fibrinogen-like domain, has several biological effects. The antibody we used in the current study recognizes the EGF-like domain and is suitable to measure total myocardial TNC level, because the subsequent fibronectin III domain contains a splice variant region that varies considerably in its expression form. The EGF-like repeat domain interacts with the epidermal growth factor receptor, and the fibronectin III-like repeat domain binds integrins to promote adhesion and mediates cell activation via toll-like receptor 4, leading to sustained inflammation.[23] The unfavourable effects of TNC on ventricular remodelling in DCM might result from ongoing myocardial damage or inability to repair the failing heart because of sustained inflammation. There is increasing evidence that splicing variants of TNC, especially those containing the fibronectin III B or C domain, may influence tissue remodelling in heart failure. Serum level, as well as cardiac tissue deposition, of the $B^+$ TNC variant was associated with mortality in DCM.[22] In patients with heart failure, serum levels of $B^+$ and $C^+$ TNC were

**Table 4 Univariable and multivariable linear regression analysis of tenascin-C (TNC) area**

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| | *β*-coefficient | *P*-value | *β*-coefficient | *P*-value |
| Age | −0.067 | 0.462 | – | – |
| Male | −0.010 | 0.911 | – | – |
| Body mass index | −0.016 | 0.862 | – | – |
| Current smoker | −0.150 | 0.097 | – | – |
| NYHA III/VI | 0.129 | 0.156 | – | – |
| Duration of heart failure | 0.266 | 0.003 | 0.117 | 0.239 |
| Systolic blood pressure | −0.088 | 0.335 | – | – |
| Diastolic blood pressure | −0.083 | 0.361 | – | – |
| Heart rate | −0.017 | 0.850 | – | – |
| Hypertension | 0.013 | 0.888 | – | – |
| Diabetes mellitus | 0.266 | 0.003 | 0.199 | 0.034 |
| Dyslipidaemia | 0.008 | 0.931 | – | – |
| Atrial fibrillation | 0.258 | 0.004 | 0.174 | 0.063 |
| Left bundle branch block | 0.117 | 0.198 | – | – |
| ICD/BiV-ICD | 0.192 | 0.033 | 0.211 | 0.015 |
| Log BNP | 0.267 | 0.003 | −0.040 | 0.735 |
| $HbA_{1c}$ | 0.055 | 0.556 | – | – |
| Haemoglobin | −0.018 | 0.847 | – | – |
| eGFR | −0.155 | 0.087 | – | – |
| Serum sodium | −0.142 | 0.118 | – | – |
| *β*-blocker on admission | 0.146 | 0.108 | – | – |
| ACE inhibitor/ARB on admission | 0.069 | 0.447 | – | – |
| Loop diuretic on admission | 0.138 | 0.129 | – | – |
| Aldosterone inhibitor on admission | 0.185 | 0.041 | −0.072 | 0.444 |
| Sulphonylurea on admission | −0.062 | 0.492 | – | – |
| Glinide on admission | −0.053 | 0.564 | – | – |
| Insulin on admission | 0.031 | 0.737 | – | – |
| *β*-blocker at discharge | 0.104 | 0.253 | – | – |
| ACE inhibitor/ARB at discharge | 0.090 | 0.320 | – | – |
| Loop diuretic at discharge | 0.181 | 0.045 | 0.180 | 0.050 |
| Aldosterone inhibitor at discharge | 0.134 | 0.140 | – | – |
| Sulphonylurea at discharge | −0.062 | 0.492 | – | – |
| Glinide at discharge | 0.143 | 0.115 | – | – |
| Insulin at discharge | −0.013 | 0.887 | – | – |
| LVEDD | 0.017 | 0.852 | – | – |
| LVESD | 0.004 | 0.961 | – | – |
| LVEF | −0.195 | 0.089 | – | – |
| RVD | 0.100 | 0.278 | – | – |
| Mitral regurgitation | 0.018 | 0.841 | – | – |
| Restrictive filling pattern | 0.215 | 0.017 | 0.099 | 0.270 |
| Mean PCWP | 0.254 | 0.005 | −0.503 | 0.032 |
| Mean PA | 0.288 | 0.001 | 0.644 | 0.008 |
| Cardiac index | −0.206 | 0.026 | 0.013 | 0.890 |
| Heart failure risk score | 0.216 | 0.017 | 0.162 | 0.093 |
| Collagen area | 0.329 | <0.001 | 0.128 | 0.209 |

TNC, tenascin-C; NYHA, New York Heart Association; ICD, implantable cardioverter defibrillator; BiV-ICD, biventricular implantable cardioverter defibrillator; BNP, brain natriuretic peptide; $HbA_{1c}$, haemoglobin $A_{1c}$; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; RVD, right ventricular diameter; PCWP, pulmonary capillary wedge pressure; PA, pulmonary artery pressure.

associated with heart chamber dilatation, physical performance, and BNP level, suggesting that they are possible biomarkers for disease severity.[24] Further immunohistochemical analysis for TNC splicing variants might provide additional valuable information related to LV remodelling.

Another intriguing finding of this study was the relevance of diabetes mellitus to myocardial TNC. Multivariable analysis showed that the presence of diabetes, as well as indices representing heart failure severity such as previous implantation of ICD or biventricular ICD, mean PCWP, and mean PA, was independently associated with myocardial TNC expression. Diabetic cardiomyopathy is a disorder of heart muscle resulting from diabetes, the pathogenesis of which is yet to be clearly defined. Remodelling of cardiac extracellular matrix is known to be a key pathological feature of diabetic cardiomyopathy. Production of matricellular proteins is increased by hyperglycaemia- and hyperinsulinaemia-induced chronic inflammation, purportedly through transforming growth factor-$\beta$ signalling.[25] In addition, persistent hyperglycaemia induces the formation of advanced glycation end-products, which is associated with increased production and reduced turnover of matricellular proteins.[26] Taking into consideration that TNC is likely to be upregulated in an inflammatory microenvironment, the increased myocardial expression of TNC in the present study might have been caused by stimulation of increased transforming growth factor-$\beta$ associated with diabetes mellitus. We believe that this study also supports the pathophysiological significance of TNC in diabetic cardiomyopathy, in that TNC may contribute to the development of worsening heart function in diabetic patients. Furthermore, from a clinical aspect, it is suggested that preventing or adequately controlling diabetes mellitus might be able to reduce myocardial chronic inflammation, diminish myocardial TNC expression and consequently promote cardiac reverse remodelling in DCM patients.

In the present study, RV endomyocardial biopsy was selected to collect myocardial tissue samples, in order to minimize the procedural risk. It is controversial as to whether RV biopsy specimens are adequate for evaluation of LV remodelling in cardiomyopathy. We consider myocardial TNC measurement using RV myocardial samples is reasonable for the present study for two reasons. First, samples were obtained from the interventricular septum, which is considered common to the right ventricle and left ventricle. Second, progression of cardiac remodelling in DCM typically results in both RV and LV dysfunction at the same time. In fact, there is evidence that right ventricular dysfunction is correlated with LV dysfunction in patients with DCM.[27] We experienced difficulty in RV volumetric and functional assessment because of geometric complexity and asymmetry of the RV.[28] Cardiac magnetic resonance imaging might be an option to evaluate RV remodelling.

There are several limitations to this study. First, this was a retrospective study that included a relatively small number of patients. Follow-up echocardiography was not performed in all patients, although 76% of the study population did undergo this procedure. Larger prospective studies with complete follow-up are needed to establish evidence for myocardial TNC expression in patients with DCM. Second, endomyocardial biopsy specimens may have some sampling errors. We used a transcatheter method to collect biopsy samples from three to five different sites in the RV septum. Evaluation of TNC accumulation was performed on all pieces collected, thus mitigating sampling error. However, there are advantages to evaluating myocardial deposition of the extracellular matrix by direct collection of myocardium by endomyocardial biopsy. Third, immunohistochemical analysis based on the use of an image processing program would be able to provide semiquantitative results at best. Future quantitative evaluation of myocardial TNC protein or mRNA would strengthen our findings. Finally, the present study, through focusing on accumulation of myocardial TNC expression, could not completely clarify the mechanisms of LV remodelling and worse outcome in DCM. Other histological approaches, for example, evaluation of expression pattern or structure of TNC are worth considering in future.

In conclusion, we have shown that myocardial TNC expression was associated with lower occurrence of LV reverse remodelling and poor long-term outcome in patients with DCM.

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