Higher Serum Tenascin-C Levels Reflect the Severity of Heart Failure, Left Ventricular Dysfunction and Remodeling in Patients With Dilated Cardiomyopathy

Fumio Terasaki, MD; Hiroshi Okamoto, MD*; Katsuya Onishi, MD**; Akira Sato, MD†; Hiroaki Shimomura, MD; Bin Tsukada, MD; Kyoko Imanaka-Yoshida, MD‡‡; Michiaki Hiroe, MD§; Toshimichi Yoshida, MD∥; Yasushi Kitaura, MD; Akira Kitabatake, MD* and Study Group for Intractable Diseases by a Grant from the Ministry of Health, Labor and Welfare of Japan

Background  Tenascin-C (TN-C), an extracellular matrix glycoprotein, is specifically expressed at high levels during embryonic development, but not in the adult heart. TN-C reappearance at sites of inflammatory tissue remodeling or wound healing under various pathologic conditions, such as acute myocardial infarction, acute myocarditis, and some cases of cardiomyopathy. Therefore, the expression of TN-C might be useful for detecting the clinical characteristics of, and ventricular remodeling in, dilated cardiomyopathy (DCM).

Methods and Results  Circulating serum TN-C levels in 107 patients with DCM were measured using an ELISA kit. Clinical data were also assessed by Pearson’s or Spearman’s correlation analysis to estimate correlations between variables. Serum TN-C levels in DCM patients were higher than those in normal controls (p<0.001). TNC levels showed a significantly positive correlation with New York Heart Association functional class (p<0.001), B-type natriuretic peptide level (p<0.001), cardiothoracic ratio on chest X-ray (p<0.01), left ventricular end-diastolic diameter (p<0.05) and left ventricular end-systolic diameter (p<0.01), and a significant negative correlation with left ventricular ejection fraction (p<0.01).

Conclusions  The findings suggest that increased serum TN-C levels indicate the severity of heart failure, left ventricular dysfunction and remodeling in patients with DCM. (Circ J 2007; 71: 327–330)

Key Words: Dilated cardiomyopathy; Heart failure; Tenascin-C; Ventricular remodeling

Dilated cardiomyopathy (DCM) refers to a spectrum of heterogeneous myocardial disorders that are characterized by ventricular dilation and depressed myocardial contractility in the absence of abnormal loading conditions or ischemic heart disease. The prognosis of DCM has improved since angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers and β-blockers have been widely used as treatment; however, despite current optimal medical management, heart failure remains a progressive disease with significant morbidity and mortality. Eventually, some cases need cardiac resynchronization or surgical interventions such as heart transplantation or left ventriculoplasty. Therefore, risk stratification in patients with DCM is receiving increasing interest, and is crucial for selection of the best treatment strategy. In particular, the diagnostic and prognostic value of B-type natriuretic peptide (BNP) and N-terminal pro-BNP have been established in heart failure patients.

Tenascin-C (TN-C), an extracellular matrix glycoprotein, plays important roles in the development of the myocardium, heart valves and coronary vessels in early-stage embryos but is not detected in adults. However, it is re-expressed under pathological conditions such as acute myocardial infarction (AMI), myocardial hibernation, myocardial injury and active myocarditis, which are closely associated with tissue injury and inflammation. Furthermore, while TN-C molecules are deposited in the local extracellular spaces, soluble forms are also released into the blood stream. For example, serum TN-C levels are significantly elevated during the acute stage after AMI, and therefore may act as a novel inflammatory marker of left ventricular (LV) remodeling and the prognosis after AMI.

We hypothesized that the TN-C levels might be useful for evaluating LV remodeling in DCM, so we measured the serum concentrations in patients with DCM, with reference to clinical characteristics and cardiac function.

Methods

Study population
A total of 107 patients (82 men, 25 women; mean age 61±14 years) with DCM, and 20 normal volunteers (14 men, 6 women; mean age 49±15 years) were enrolled. Patients with DCM were admitted or followed up at Osaka Medical College Hospital, Hokkaido University Hospital, and Cardiology, International Medical Center of Japan, Tokyo, Japan
Mie University Hospital, and Yokosuka Kyosai Hospital, Japan, between January 2001 and March 2005. The clinical diagnosis of DCM was made according to the World Health Organization/International Society and Federation of Cardiology task force1 and the clinical characteristics are shown in Table 1. Written informed consent was given by all patients and volunteers, and the study protocol was approved by the institutional review board.

**Clinical Information**

Age, gender, systolic and diastolic blood pressures (mmHg), heart rate (beats/min) and New York Heart Association (NYHA) functional class were evaluated as essential information. Cardiotoracic ratio (CTR) was examined on chest X-ray. LV end-diastolic diameter (LVDd) and end-systolic diameter (LVDs), LV ejection fraction (LVEF) were measured by standard echocardiography.

**Assay of Serum TN-C Levels by ELISA**

Blood samples were centrifuged at 15,000G for 15 min, and the resulting supernatants were stored at –80°C until

---

**Table 1 Clinical Characteristics of Patients With Dilated Cardiomyopathy (n=107)**

| Age (years) | 61±14 |
| Men | 82 (77%) |
| New York Heart Association functional class | |
| I | 16 (15%) |
| II | 48 (45%) |
| III | 31 (29%) |
| IV | 12 (11%) |
| Medication | |
| Angiotensin-converting enzyme inhibitors | 61 (57%) |
| Angiotensin II receptor antagonists | 34 (32%) |
| Calcium channel antagonists | 12 (11%) |
| β-blockers | 67 (63%) |
| Diuretics | 75 (70%) |
| Digitalis | 44 (41%) |
| Systolic blood pressure (mmHg) | 116±21 |
| Diastolic blood pressure (mmHg) | 68±12 |
| Heart rate (beats/min) | 74±13 |
| Left ventricular end-diastolic diameter (mm) | 62±9 |
| Left ventricular end-systolic diameter (mm) | 51±11 |
| Left ventricular ejection fraction (%) | 37±14 |
| Cardiotoracic ratio (%) | 57±8 |

Data are means±SDs or number (%).

---

**Fig 1. Associations of serum tenascin-C (TN-C) concentration with clinical information. BNP, B-type natriuretic peptide; CTR, cardiotoracic ratio; EF, ejection fraction; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; NYHA, New York Heart Association.**
analysis. Serum levels of TN-C with the large subunit containing the C domain of FNIII repeats were determined using an ELISA kit with 2 monoclonal antibodies, 4F10TT and 19C4MS (IBL, Gunma, Japan), as previously described.20

**Biochemical Analysis**

Plasma BNP concentrations were measured using a specific immunoradiometric commercial assay kit (Shionogi, Japan). Serum C-reactive protein (CRP) concentrations were measured by latex agglutination immunophotometric assay.

**Statistical Analysis**

Data are expressed as means±SD for continuous variables, and as numbers (percentages) for categorical variables. Continuous variables were analyzed by the unpaired Student’s t-test. Pearson’s or Spearman’s correlation analysis was performed to estimate correlations between variables. A p-value <0.05 was considered statistically significant.

**Results**

Serum TN-C levels were significantly higher in patients with DCM than in normal volunteers (73.3±35.1 vs 30.9±8.8 ng/ml, p<0.001). Serum TN-C levels increased according to NYHA functional class (r=0.51, p=0.0001). Furthermore, the levels were negatively correlated with LVEF (r=-0.27, p=0.006), and positively correlated with LVDD (r=0.20, p=0.04), LVDs (r=0.27, p=0.005), CTR (r=0.32, p=0.002) and plasma BNP level (r=0.44, p=0.0001) (Fig 1).

However, serum CRP levels did not correlate with CTR, NYHA functional class, LVEF, LVDD, and LVDs on echocardiography, plasma BNP levels, and serum TN-C levels.

**Discussion**

In the present study, serum TN-C levels were significantly higher in 107 patients with DCM than in 20 normal volunteers. Among the DCM patients, serum TN-C levels showed significant positive correlation with NYHA functional class, CTR, LVDD, LVDS and plasma BNP levels, and negative correlation with LVEF, indicating that the level reflects the severity of heart failure, LV dysfunction and remodeling. Our multicenter study with a larger DCM population has confirmed previous preliminary results obtained from 31 cases of DCM,21 and further showed a clear association between higher TN-C levels and LV remodeling, as well as LV dysfunction.

Several lines of evidence suggest that inflammation plays a pathogenic role in the development and progression of congestive heart failure, influencing cardiac contractility and myocardial remodeling.22-24 In fact, pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, are known to be upregulated in the myocardium and blood stream of DCM patients, and their high expression levels correlate to progression of refractory heart failure leading to morbidity and mortality.25,26 It is postulated that these cytokines expressed in the failed myocardium may lead to the necrosis/apoptosis and hypertrophy of cardiomyocytes, and alter the nature of the supporting connective tissue by activating matrix metalloproteinases.

As previously reported, TN-C is specifically expressed in the myocardial inflammatory lesions of myocarditis16-18 and AMI12,15 Furthermore, serum TN-C levels are elevated in AMI patients, reflecting local expression of TN-C in the infarcted myocardium.19 Therefore, increased serum TN-C levels in DCM patients suggest the involvement of inflammation in the myocardium. Supporting this possibility, we have recently found that most of the myocardium in DCM patients shows varying degrees of inflammation, and that expression of TN-C is enhanced in the areas of active inflammation with local tissue remodeling (unpublished data). Furthermore, after AMI, patients with high serum levels of TN-C are at higher risk of major adverse cardiac events, which suggests that the expression level of TN-C reflects remodeling activity.19 Therefore, TN-C expression might also reflect long-term cardiac function and prognosis in DCM patients. In this study, serum CRP levels were not associated with the parameters of LV size, function or serum TN-C levels (p=0.0942), which suggests that circulating CRP levels may not reflect the severity of heart failure, LV remodeling or myocardial inflammation in patients with DCM.

The origin of the elevated level of circulating TN-C in DCM is a matter of interest. It has been reported that expression of TN-C is observed in endomyocardial biopsy specimens of patients with DCM,17 which strongly suggests that the serum TN-C arises from heart tissue. In the pathological myocardium, results obtained from experimental animal models, as well as human myocarditis samples, have identified that the major source of TN-C is interstitial fibroblasts.13,16,18 Various cytokines, growth factors, hypoxia, mechanical stress, acidosis, and angiotensin II, which are important mediators of myocardial injury and inflammation during progression of heart failure in DCM, stimulate cardiac fibroblasts to synthesize TN-C in vitro.28 In addition, endothelial cells and vascular smooth muscle cells of various organs have the potential to synthesize TN-C under these same stimulations.10,29 The elevated levels of circulating soluble inflammatory mediators in heart failure patients might also stimulate endothelial cells of, for example, the liver, or lung, to secrete TN-C into the blood stream.

**Study Limitations**

Evaluation of the relationship between serum TN-C levels and the prognosis or adverse cardiac events was not conducted satisfactorily, in part because of the relatively short follow-up period. Further study is warranted to examine whether circulating TN-C levels reflect morbidity or mortality in DCM patients. Inflammatory cytokines, such as TNF-α and IL-6, were not measured in enough patients in this study, partly because of the limitations of a multicenter study.

**Conclusions**

Our present data suggest that increased serum TN-C levels are associated with the severity of heart failure and the LV dysfunction and remodeling in patients with DCM. Further research is needed to discover whether TN-C levels are a promising biomarker for determining prognosis and evaluating therapeutic efficacy in patients with DCM.

**Acknowledgments**

This study was supported in part by a research grant for intractable diseases from the Ministry of Health, Labor and Welfare of Japan.

**References**

1. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B,


