TN-C levels in AMI patients (maximum, 85.9 ± 41.7 ng/ml) were significantly higher than in OMI (27.4 ± 11.7 ng/ml, $p < 0.01$) and control patients (30.9 ± 8.8 ng/ml, $p < 0.01$, Fig. 2). TN-C levels were not statistically distinguishable between control and OMI patients. While CK-MB levels peaked within 12 h and rapidly decreased within 5 days following infarction, serum TN-C levels were elevated on admission (63.3 ± 30.1 ng/ml), peaked at day 5 (83.2 ± 43.0 ng/ml) then gradually decreased, but remained elevated at day 28 (51.8±17.8 ng/ml, Fig. 3).

Comparison of Clinical Characteristics and Left Ventricular Parameters between Remodeling and Non-Remodeling Groups

Out of 105 patients, 25 (23.8 %) showed LV remodeling at 6 months. During the follow-up period (mean: 43.9±19.6 months), 15 MACE (14.3 %) including 8 deaths and 7 hospitalizations for worsening heart failure were observed. Incidence of MACE in the LV remodeling group was higher (12/25) than in the non-remodeling group (3/80, $p < 0.01$).

Clinical characteristics and left ventricular parameters of the study patients are shown in Table 2. There were no significant differences in age, perfusion time, infarct location, systolic blood pressure, and use of cardiovascular medications between the two groups. Peak TN-C levels were significantly higher in the remodeling group than the non-remodeling group (112 ± 37 vs 66 ± 29, $p < 0.0001$). Peak CK-MB,

LVESV and total defect scores on admission, and BNP levels on days 5 and 28 after onset of AMI were also significantly higher in the remodeling group than the non-remodeling group, while LVEF on admission was significantly lower in the remodeling group. No siginificant relationship was found between peak TN-C and peak CK-MB or total defect socre.

ROC Analysis of Clinical Variables for Predicting LV Remodeling and MACE

We performed ROC analysis of the following clinical variables: peak serum TN-C levels and plasma BNP levels on days 5 and 28 after AMI, and peak CK-MB, LVEDV, LVESV, and LVEF on admission for prediction of LV remodeling and MACE (Table 3). For prediction of LV remodeling, the AUC of the peak serum TN-C level was 0.849, and highest among the analyzed variables. The best cut-off value of serum TN-C for prediction of LV remodeling was 84.8 ng/ml, with a sensitivity of 84%, specificity of 77 %, and accuracy of 80 %.

Comment [FEC1]: Is this your intended meaning?

For prediction of MACE, the AUC of the peak serum TN-C level was 0.788, which was also higher than any other variables. The best cut-off value for prediction of MACE was 92.8 ng/ml, with a sensitivity of 73 %, specificity of 80 % and accuracy of 78 %. The AUC of plasma BNP on day 28 was also high for prediction of MACE (0.783). The best cut-off value of BNP level was 163 pg/ml with a sensitivity of 80 %, specificity of 59 %, and accuracy of 70 %.

Comment [FEC2]: As above

Univariate and Multivariate Predictors of MACE

Table 4 shows the results of univariate and multivariate Cox proportional hazards model analyses between 10 variables related to MACE. In the univariate analysis, the peak TN-C level, plasma BNP level on days 5 and 28, LVESV, LVEF, and total defect scores on admission were predictive factors. According to multivariate analysis, peak TN-C level was the most important independent predictor of MACE during a follow-up period of up to 5.5 years after infarction. Plasma BNP level on day 28 was also a significant predictor of MACE.

Survival Curves obtained by Kaplan-Meier Analysis

During the follow-up period (mean: 43.9±19.6 months), there were 5 deaths and 5 hospitalizations for worsening heart failure in patients with TN-C $\geq$ 92.8 ng/ml, and 3 deaths and 2 hospitalizations for worsening heart failure in patients with TN-C < 92.8 ng/ml. Fig. 4 shows Kaplan-Meier MACE free survival curves comparing increased risks of death and hospitalization between patients with TN-C $\geq$ 92.8 ng/ml and those with TN-C < 92.8 ng/ml ($p < 0.0001$).

DISCUSSION

Major novel findings in the present study were as follows: (1) serum TN-C levels were significantly elevated during acute stages after AMI; (2) TN-C levels were significantly higher in the LV remodeling group than the non-remodeling group; and (3) AMI patients with high TN-C levels were at much higher risk of MACE for up to 5 years. Thus, serum TN-C levels in acute stages following AMI might be a predictive biomarker of LV remodeling during the recovery phase and prognosis.

Elevated serum TN-C levels in AMI patients

Using rat and mouse myocardial infarction models, we previously reported that TN-C was synthesized during acute stages by interstitial fibroblasts in the border zone myocardium surrounding infarcted lesions (8), and could play several important roles in myocardial repair (8,14,21). In the present paper, we demonstrated that serum TN-C levels in AMI patients were significantly higher than those in OMI patients and controls. Immunostaining of autopsied specimens confirmed expression of TN-C in human myocardium in acute stages following infarction, while no expression was detected in normal myocardium or in scar tissues of OMI patients. Therefore, TN-C synthesized in infarcted myocardium could enter the bloodstream and cause elevation of serum TN-C levels in AMI patients. In various tissue injuries, TN-C molecules are synthesized by interstitial cells residing in injured sites. While molecules are deposited in extracellular spaces and regulate cell behavior in the local environment, soluble forms might also be released into body fluids. For example, TN-C levels in synovial fluid from patients with osteoarthritis(16),and aseptic loosening after arthroplasty (22), and in serum of patients with hepatic fibrosis (23) are reportedly increase in correlation with disease activity.

In our AMI patients, significantly elevated TN-C levels were noted within 24 h after onset. Levels peaked at day 5 and then gradually decreased. This time course of serum TN-C levels was previously shown to correspond to local expression of TN-C in infarcted myocardia of humans (9) and rats (8), as detected by immunohistochemistry. It is noteworthy that the peak of serum TN-C occurred later than that for CK-MB, and persisted much longer. Furthermore, peaks of TN-C did not significantly correlate with total defect scores on myocardial SPECT with ^{99m}Tc-tetrofosmin, or with peaks of CK-MB. These results indicate that elevation of TN-C levels might not directly reflect

cardiomyocyte death. TN-C synthesis by cardiac fibroblasts is stimulated by various cytokines, growth factors, hypoxia, acidosis, mechanical stress, and angiotensin II (21), which could be closely related to myocardial injury and inflammation during the wound healing process. Therefore, peak TN-C levels could reflect activity of interstitial cells during tissue remodeling after injury rather than damage of cardiomyocytes.

TN-C as a Marker for LV Remodeling and Long-term Clinical Outcomes

Most importantly, patients with LV remodeling showed higher peak TN-C levels than patients with non-LV remodeling, and patients with higher peak TN-C levels had a greater incidence of MACE and worse long-term prognosis. A previous report revealed that patients with significant LV remodeling 6 months after infarction had worse long-term clinical outcomes (2). Since TN-C levels peaked within 1 week after infarction, our results suggest that TN-C could be an early predictive marker for future ventricular remodeling.

One of the major determinants of ventricular remodeling following AMI could be infarct size (2,24). Therefore, myocyte injury markers such as cardiac troponin I and T, creatine kinase (CK), and CK isoforms appear to be useful in predicting late ventricular dilatation (2). It was also suggested that the systemic inflammatory marker CRP (25,26), and neurohormones secreted by cardiomyocytes including ANP (27) and BNP (28) are further biomarkers of ventricular remodeling. A recent report suggested that plasma BNP levels at 3 to 4 weeks after AMI could be independent predictors of cardiac death (29). In the present study, our analysis of the prognostic value of various clinical variables also supported the possibility that large infarction and high plasma BNP levels might predict MACE.

LV remodeling involves multi-step reactions, which orchestrate structural alternation and rearrangement of cells, and connective tissues. During these processes, disproportionate activation of matrix metalloproteinases (MMPs) has recently received increasing attention in progression of unfavorable tissue remodeling (30-33). Several reports have suggested that deletions of MMP2 and MMP9 attenuate ventricular remodeling (34-36), and that MMPs could act as biomarkers of ventricular remodeling (37-39).

TN-C has many biological effects, including regulation of cell activity during early stages of tissue repair (4,8,11,14,40). It up-regulates MMP expression in a number of cell types (41,42) and inhibits strong linkages between cardiomyocytes and connective tissues (8,21). Therefore, excessive amounts of TN-C might cause disproportionate MMP activation, which, in turn, would lead to progressive degradation of connective tissues, and slippage of myocytes within the LV wall, finally resulting in LV wall thinning and dilatation. On the other hand, TN-C also has the potential to promote myocardial repair and prevent ventricular dilatation by recruitment of myofibroblasts and enhancement of collagen fiber contraction (14,43). Thus, the effects of TN-C on ventricular remodeling are not simple, but rather are bidirectional. In the present study, we found that high levels of serum TN-C could be related to a greater incidence of ventricular remodeling and poor prognosis, suggesting that excessive and sustained increments of TN-C could cause inappropriate reconstruction of infarcted ventricular walls.

Clinical implications and limitations

This preliminary study suggests that serum TN-C might be a novel marker reflecting active structural remodeling in the myocardium following infarction, with high TN-C levels at acute stages possibly predicting progression of LV remodeling.

However, despite the findings, the current study has some limitations. First, the sample size was relative small. Second, prognosis of our AMI patients receiving primary coronary angioplasty was good, and there were only five deaths and four hospitalizations due to heart failure out of 105 patients during the follow-up period of 3 to 5 years. Further large-scale prospective investigations and careful comparisons with other clinical parameters are therefore required to confirm the predictive ability of TN-C in LV remodeling and MACE.

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Figure legends

Fig. 1. Representative microscopic images of TN-C expression in autopsied human myocardia from a normal case (A, D, G), an AMI patient 36 h after myocardial infarction (B, E, H), and an OMI patient (C, F, I). (A, B, C) H & E staining; (D, E, F) immunolabeling with antibody clones 4F10TT (G, H, I) and 6C6MS. Positive immunostainings with the two monoclonal antibodies were observed in the infarcted myocardial lesion of the AMI patients. Bar = 100 μm.

Fig. 2. Comparisons of serum TN-C levels between AMI, OMI, and control cases. n.s = not significant.

Fig. 3. Serial changes in serum TN-C and CKMB levels in patients with AMI. Values represent the mean and standard error. Serum TN-C levels were elevated on admission, peaked at day 5, and then gradually decreased (A). In contrast, CK-MB levels peaked within 12 h, and then rapidly decreased (B).

Fig. 4. Kaplan-Meier survival curves for the study population

Elevated TN-C levels significantly increased risks of death and hospitalization ($p < 0.0001$).

Table 1. Clinical Characteristics of 105 Patients with AMI Undergoing Successful Primary PCI

Age (y)	66 ± 12
Male (%)	70
Risk factors	
Current smoker (%)	53
Diabetes (%)	15
Hypertension (%)	39
Hyperlipidemia (%)	48
Infarct location	
Anterior (%)	54
Inferior (%)	26
Lateral (%)	20
Reperfusion times (h)	6.4 ± 4.7

Table 2. Clinical Characteristics of Patients in the Remodeling and Non-remodeling groups

	Non-remodeling (n = 80)	Remodeling (n = 25)	p Value
Age (y)	66.0 ± 12.3	64.6 ± 11.1	0.401
Anterior MI (%)	48	62	0.361
Reperfusion times (h)	6.4 ± 5.1	6.6 ± 4.0	0.476
SBP (mmHg)	122 ± 15	117 ± 15	0.436
Peak TNC (ng/ml)	66 ± 29	112 ± 37	< 0.0001
Peak CK-MB (IU/l)	250 ± 149	419 ± 227	0.0011
BNP on day 5 (pg/ml)	104 ± 73	190 ± 91	< 0.0001
BNP on day 28 (pg/ml)	97 ± 66	233 ± 198	0.0003
Left ventricular			
EDV on admission (ml)	93 ± 25	109 ± 33	0.051
ESV on admission (ml)	45 ± 17	60 ± 22	0.013
EF on admission (%)	52 ± 11	46 ± 7	0.033
TDS on admission	13.2 ± 6.8	19.1 ± 4.5	0.0001
Drugs			
ACE inhibitors	57 %	73 %	0.172
ARB	23 %	12 %	0.24
Ca antagonists	21 %	19 %	0.899
Diuretics	21 %	34 %	0.205
Beta-blockers	31 %	27 %	0.738

Values are means ± SD. SBP = systolic blood pressure, EDV = end-diastolic volume;
ESV = end-systolic volume; EF = ejection fraction, TDS = total defect score;
ARB = angiotensin II receptor blocker

Table 3. Prognostic Value of each Clinical Variable in Predicting LV Remodeling and Cardiac Events According to ROC Analysis

	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Accuracy (%)
LV remodeling					
Peak Tenascin-C (ng/ml)	0.849	84.8	84	77	80
BNP on day 5 (pg/ml)	0.817	138	92	71	79
BNP on day 28 (pg/ml)	0.769	143	76	67	70
Peak CK-MB (IU/l)	0.743	322	76	71	73
LVEDV (ml)	0.646	101	64	70	67
LVESV (ml)	0.684	53	72	57	63
LVEF (%)	0.706	48	76	57	64
Cardiac events					
Peak Tenascin-C (ng/ml)	0.788	92.8	73	80	78
BNP on day 5 (pg/ml)	0.760	148	80	59	64
BNP on day 28 (pg/ml)	0.783	163	80	65	70
Peak CK-MB (IU/l)	0.669	335	73	67	67
LVEDV (ml)	0.640	102	60	62	61
LVESV (ml)	0.686	55	73	54	59
LVEF (%)	0.689	47	67	60	62

AUC = area under the ROC curve; LVEDV = left ventricular end-diastolic volume

LVESV = left ventricular end-systolic volume; EF = ejection fraction

Table 4. Univariate and Multivariate Analyses of the Value of Each Variable in Predicting Cardiac Events

Predictive Factors During the Acute Phase	Univariate		Multivariate	
	X^2	P	X^2	P
Age	3.13	0.076		
Reperfusion time	1.38	0.238		
Peak CK-MB	3.01	0.082		
Peak Tenascin-C	14.8	0.0001	10.82	0.001
BNP on day 5	4.32	0.037		
BNP on day 28	7.94	0.004	4.23	0.039
LVEDV	3.61	0.057		
LVESV	8.76	0.003	0.36	0.543
LVEF	6.96	0.008	0.76	0.381
Total Defect Scores	6.51	0.011	0.03	0.852

LVEDV = left ventricular end-diastolic volume; EF = ejection fraction

LVESV = left ventricular end-systolic volume