PRELIMINARY REPORT

Measuring serum aminoterminal type III procollagen peptide, 7S domain of type IV collagen, and cardiac troponin T in patients with idiopathic dilated cardiomyopathy and secondary cardiomyopathy

Yukihito Sato, Kazuaki Kataoka, Akira Matsumori, Shigetake Sasayama, Tasuku Yamada, Haruyasu Ito, Yoshiki Takatsu

Abstract

Objective—To identify new prognostic indicators in idiopathic dilated cardiomyopathy (DCM) and secondary cardiomyopathy.

Design and patients—Serum concentrations of aminoterminal propeptides of type III procollagen and the 7S domain of type IV collagen (7S collagen)—which have recently been used as indicators of collagen matrix turnover in other diseases—and of cardiac troponin T were measured in 17 consecutive patients with DCM and in four patients with secondary cardiomyopathy (one associated with hyperthyroidism, two with chronic renal failure, one with amyloidosis), confirmed by endomyocardial biopsy. The correlation of these variables with short term prognosis was then assessed prospectively. **Results**—11 of the patients were positive for type III procollagen, 7S collagen, or troponin T even though their creatine kinase concentrations were within the normal range. These patients had a poor short term prognosis ($p < 0.001$).

Conclusions—Within the DCM and secondary cardiomyopathy groups, there was a subgroup of patients with raised concentrations of serum collagen and troponin T, for whom short term prognosis was poor. Although it is unclear whether these serum peptide levels reflect ongoing myocyte degeneration and interstitial fibrosis, they may serve as useful new prognostic indicators for cardiomyopathy.

(Heart 1997;78:505–508)

Keywords: troponin T; collagen; dilated cardiomyopathy

Idiopathic dilated cardiomyopathy (DCM) is a primary myocardial disease of unknown cause characterised by ventricular dilatation and impaired myocardial contractility.¹ Although

characteristics such as ventricular enlargement, New York Heart Association (NYHA) functional class, and haemodynamic abnormalities are helpful in determining the risk of cardiac events, the assessment of prognosis for an individual patient with cardiomyopathy remains difficult,¹ so a more accurate marker reflecting the pathogenesis of DCM is needed.

Microscopic changes associated with DCM and secondary cardiomyopathy can be roughly divided into interstitial changes and myocyte degeneration. Although there has been little study of interstitial matrix turnover and ongoing myocyte degeneration in patients with cardiomyopathy, serum aminoterminal propeptides of type III procollagen (type III procollagen) and the 7S domain of type IV collagen (7S collagen) have recently been used as indicators of collagen matrix turnover in other diseases,² while cardiac troponin T is thought to reflect myocyte degeneration. In this study, serum concentrations of type III procollagen, 7S collagen, and troponin T were measured, and correlations between these variables and cardiac event prognosis were assessed prospectively in patients with DCM and secondary cardiomyopathy.

Methods

Seventeen consecutive patients with DCM and four with secondary cardiomyopathy (one associated with hyperthyroidism, two with chronic renal failure, and one with amyloidosis) admitted to Hyogo Prefectural Amagasaki Hospital between September 1995 and February 1997 were included in the study. Cardiac catheterisation including coronary angiography, left ventriculography, haemodynamic studies, and endomyocardial biopsy was performed in all patients. No significant coronary stenosis was found in any of them. The criteria used for the diagnosis of DCM were based on the definition of the WHO/ISFC task force.³ None of our DCM patients had a history of infective myocarditis, metabolic disease,

Division of Cardiology,
Department of
Internal Medicine,
Hyogo Prefectural
Amagasaki Hospital,
Amagasaki, Hyogo,
Japan

Y Sato
K Kataoka
T Yamada
H Ito
Y Takatsu

Department of
Cardiovascular
Medicine, Graduate
School of Medicine,
Kyoto University,
Kyoto, Japan
A Matsumori
S Sasayama

Correspondence to:
Dr Sato, Division of
Cardiology, Department of
Internal Medicine, Hyogo
Prefectural Amagasaki
Hospital, 1-1-1
Higashidaimotsu-cho,
Amagasaki, Hyogo 660,
Japan.

Accepted for publication
22 July 1997

Table 1 Clinical data and diagnosis

Patient No	Age (years)	Sex	Diagnosis	NYHA	AST (IU/l)	ALT (IU/l)	Creatinine (μ mol/l)	BUN (mmol/l)
1	43	M	DCM	II	25	12	70.7	5.0
2	73	F	DCM	II	19	17	53.0	6.5
3	19	M	DCM	II	33	42	61.9	4.0
4	86	F	DCM	III	20	10	114.9	10.4
5	70	M	DCM	II	31	26	79.6	7.6
6	48	F	DCM	III	34	25	79.6	5.8
7	39	M	Hyperthyroidism	II	19	41	61.9	5.4
8	49	M	DCM	II	44	38	61.9	5.8
9	64	F	DCM	II	18	6	53.0	5.8
10	55	M	Amyloidosis	II	35	13	79.6	5.4
11	75	M	DCM	II	14	10	150.3	9.7
12	47	F	DCM	II	26	18	61.9	6.1
13	57	F	DCM	II	25	10	61.9	6.1
14	51	F	DCM	III	29	17	53.0	6.5
15	69	M	Chronic renal failure	III	36	22	327.1	28.1
16	31	M	Chronic renal failure	II	14	16	839.8	16.6
17	55	F	DCM	II	23	22	44.2	5.4
18	51	M	DCM	II	35	38	44.2	5.4
19	58	M	DCM	II	19	12	61.9	5.0
20	49	M	DCM	II	32	30	44.2	5.4
21	55	M	DCM	II	36	41	53.0	5.8

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; DCM, dilated cardiomyopathy; NYHA, New York Heart Association functional class.

Table 2 Serum concentrations of type III procollagen peptide (P III P), 7S domain of type IV collagen (7S collagen), and cardiac troponin T (TnT)

Patient No	CK (IU/ml)	P III P (U/ml)	7S collagen (ng/ml)	TnT (ng/ml)	LVEDP (mm Hg)	CI (l/min/m ²)	LVEDVI (ml/m ²)	EF (%)
1	143	0.5	4.6	<0.10	11	3.62	85	40
2	42	0.7	4.2	<0.10	14	2.21	93	17
3	156	0.6	2.8	<0.10	23	3.32	149	53
4	31	1.6	2.9	0.15	18	1.84	83	47
5	52	0.6	3.3	<0.10	10	2.47	80	24
6	39	0.6	5.7	0.22	16	2.07	113	24
7	124	0.8	3.9	<0.10	20	6.43	105	69
8	76	1.0	5.1	0.27	12	2.70	96	45
9	73	0.7	3.9	<0.10	16	3.39	124	51
10	123	0.6	6.5	0.26	17	2.67	113	58
11	51	1.1	3.8	<0.10	15	2.01	122	39
12	99	0.8	9.1	<0.10	12	1.93	105	51
13	40	0.8	4.5	<0.10	9	2.91	100	49
14	125	0.6	8.2	<0.10	48	2.78	76	36
15	84	0.8	7.1	0.39	31	1.85	145	26
16	106	1.8	3.4	<0.10	25	5.47	190	40
17	68	0.4	6.4	<0.10	26	3.28	187	9
18	32	0.6	4.9	<0.10	23	3.62	129	45
19	119	0.4	4.7	<0.10	9	3.38	104	52
20	76	0.6	6.8	<0.10	9	2.08	106	26
21	86	0.6	4.3	<0.10	12	2.91	113	30

Eleven of the 21 patients had significantly raised serum concentrations (shown in bold) of P III P, 7S collagen, or TnT, even though their CK values were within the normal range. CK, creatine kinase; LVEDP, left ventricular end diastolic pressure; CI, cardiac index; LVEDVI, left ventricular end diastolic volume index; EF, left ventricular ejection fraction.

general systemic disease, hereditary disease or sensitivity, or any of the toxic reactions listed in the definition. Secondary cardiomyopathy due to hyperthyroidism was diagnosed by abnormal thyroid function tests and by the improvement in symptoms with treatment of the thyroid disorder. Secondary cardiomyopathy due to chronic renal failure was diagnosed by the presence of systolic dysfunction not observed before renal function impairment. The diagnosis of amyloidosis was based on the presence of myocardial amyloid deposits. More than three endomyocardial biopsy specimens were obtained from each patient. Between three and five specimens per patient were stained with haematoxylin and eosin. Microscopy revealed combinations of myocyte hypertrophy, nuclear hypertrophy, myocyte degeneration with myofibrillar attenuation, and interstitial and perivascular fibrosis in all patients with DCM

and secondary cardiomyopathy. Histological examination of the amyloid also showed myocyte degeneration and interstitial fibrosis. Definite myocarditis was excluded on the basis of the Dallas criteria.⁴

Blood samples were taken during the patients' first admission. Measurements of creatine kinase, creatinine, blood urea nitrogen, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were carried out as routine analyses. Serum samples for measurements of type III procollagen, 7S collagen, and troponin T were stored at -20°C until the assay. Commercially available radioimmunoassay kits were used for assays. Positive patients were defined as those with concentrations greater than 0.8 U/ml for type III procollagen, 5.0 ng/ml for 7S collagen, and 0.1 ng/ml for troponin T (first generation).

Cardiac event-free rates were estimated with the Kaplan-Meier method. Cardiac events were taken to be episodes of heart failure requiring the addition of diuretic treatment or hospital admission, or cardiac death.

Results

The clinical characteristics of the patients are listed in table 1. None of the patients with DCM had significant liver or renal disease. Patients with cardiomyopathy secondary to chronic renal failure had raised serum concentrations of blood urea nitrogen and creatinine.

Of the 21 consecutive DCM and secondary cardiomyopathy patients, 11 were positive for type III procollagen, 7S collagen, or troponin T (table 2). None of these patients had significantly raised serum creatine kinase. There was no correlation between haemodynamic variables and serum collagen concentrations.

Clinical follow up was performed for all 21 patients. Patients were treated with conventional diuretics, digoxin, or vasodilators. Use of angiotensin converting enzyme inhibitors did not appear to affect short term prognosis in this small group of patients. Patient 4 died unexpectedly six months after first being admitted to our hospital. Patient 6 developed heart failure requiring the addition of diuretic treatment after four months and was readmitted to hospital 16 months after her first admission. Patient 8 developed heart failure and required diuretic treatment and hospital admission after five months. Patient 10, with amyloidosis, died of cardiogenic shock nine months after his first admission. Patient 11 was admitted to hospital three times during the previous 12 months. Patient 14 died after seven months, just after ascending the stairs in her house. Patient 15, with cardiomyopathy secondary to chronic renal failure, was admitted to our hospital for heart failure three times in the previous six months.

The 10 patients who were seronegative for type III procollagen, 7S collagen, and troponin T were stable during the follow up period and required no additional drug treatment. The cardiac event-free rate for these seronegative patients was significantly higher than for the seropositive patients on day 216 ($p < 0.001$) (fig 1).

Figure 1 Rate of freedom from cardiac events. The event-free rate in patients seronegative for type III procollagen, 7S collagen, or troponin T was significantly higher than in seropositive patients on day 216 ($p < 0.001$).

Discussion

DCM and secondary cardiomyopathy are characterised histologically by myocyte degeneration and interstitial changes. Although extracellular matrix turnover and ongoing myocyte degeneration in DCM have yet to be established and characterised, we measured serum type III procollagen and 7S collagen—recently developed markers of collagen matrix turnover, especially in patients with liver disease—along with serum troponin T concentrations in this study of patients with primary and secondary cardiomyopathy. Within the DCM and secondary cardiomyopathy groups, there was a subgroup of patients whose serum peptide concentrations were raised and for whom the short term prognosis was poor.

The procollagen type III aminoterminal peptide is an extension peptide of collagen type III. During the conversion of procollagen type III to collagen type III, type III procollagen is cleaved in a stoichiometric fashion and liberated into extracellular fluid. Type IV collagen, which is one of the major constituents of basement membranes and fibrous tissue, is composed of a 7S collagen domain formed by four aminoterminal ends linked together in an antiparallel arrangement, and a globular carboxyterminal cross linking domain.

Raised serum type III procollagen concentrations have been reported in patients with acute myocardial infarction⁵ and essential hypertension.⁶ In our study, 11 patients were positive for type III procollagen or 7S collagen.

These peptides are not tissue specific, and their cardiac origin remains speculative. However, none of our patients with DCM had significant lung, liver, or renal disease, nor was any relation found between pulmonary capillary wedge pressure, liver enzymes, serum creatinine, or blood urea nitrogen concentration and changes in serum type III procollagen (data not shown). It is unlikely that lung, liver, or kidney is the source of extracellular matrix proteins in our patients with DCM. Whether the serum levels of type III procollagen or 7S collagen reflect collagen matrix turnover is still unclear. However, we recently investigated a case of acute myocarditis following common cold symptoms with raised serum troponin T in the acute stage and raised serum type III procollagen in the chronic stage. Further studies are needed to determine the pathologi-

cal significance of serum type III procollagen and 7S collagen in patients with cardiomyopathy.

The determination of serum creatine kinase concentration is a well established and widely accepted method for the laboratory diagnosis and follow up of myocardial infarction. Troponin T is a tropomyosin binding protein of the troponin regulatory complex located on the thin myofilament of the contractile apparatus. In our study, no patients with DCM or secondary cardiomyopathy were positive for creatine kinase. However, five patients were positive for troponin T. We previously demonstrated indium-111 antimyosin antibody uptake in patients with DCM, suggesting ongoing myocyte degeneration.⁷ Interestingly, the son of patient 6 was recently diagnosed as having hypertrophic cardiomyopathy, and patient 6 herself may have end stage hypertrophic cardiomyopathy resembling DCM. A rise in serum troponin T in patients with cardiomyopathy may suggest ongoing myocyte degeneration.

Follow up serum samples were obtained from several patients. Patient 6 remained positive for serum troponin T (0.11 ng/ml) after 11 months. Patient 8 remained positive for serum type III procollagen (0.9 U/ml), 7S collagen (6.7 ng/ml), and troponin T (0.24 ng/ml) after six months. The serum troponin T concentration in patient 12 was increased, at 0.15 ng/ml, after seven months. Patient 15 was positive for serum type III procollagen (1.1 U/ml), 7S collagen (5.1 ng/ml), and troponin T (0.12 ng/ml) after two months.

We cannot rule out the possibility of biopsy sample error, with failure to diagnose chronic persistent or recurrent myocarditis. However, in the myocarditis treatment trial, few patients showed evidence of ongoing or recurrent myocarditis on the basis of the Dallas criteria.⁸ It is unlikely that there were several cases with chronic persistent or recurrent myocarditis in this small group of consecutive patients. Follow up serum samples from patient 1 (after eight months), patient 2 (after 12 months), patient 9 (after seven months), patient 19 (after two months), and patient 21 (after two months) remained negative for these peptides.

CONCLUSIONS

In summary, our prospective study revealed a subgroup of patients with DCM or secondary cardiomyopathy with raised serum type III procollagen, 7S collagen, or troponin T, whose short term prognosis was poor. It was notable that some patients had significant serum levels of troponin T, even though their creatine kinase was within the normal range. These serum peptides may be useful additional prognostic indicators in patients with cardiomyopathy.

- 1 Dec GW, Fuster V. Idiopathic dilated cardiomyopathy. *N Engl J Med* 1994;331:1564–75.
- 2 Risteli L, Risteli J. Noninvasive methods for detection of organ fibrosis. In: Rojkind M, ed. *Connective tissue in health and disease*, vol 1. Boca Raton: CRC Press, 1990:61–98.
- 3 Brandenburg RO, Chazov E, Cherian G, Falase AO, Grossogeat Y, Kawai C, et al. Report of the WHO/ISFC task force on definition and classification of cardiomyopathies. *Circulation* 1981;64:437–8A.

- 4 Aretz HT, Billingham ME, Edwards WD. Myocarditis: a histopathologic definition and classification. *Am J Cardiovasc Pathol* 1986;**1**:3–14.
- 5 Jensen LT, H-Petersen K, Toft P, Bentsen KD, Grande P, Simonsen EE, et al. Serum aminoterminal type III procollagen peptide reflects repair after acute myocardial infarction. *Circulation* 1990;**81**:52–7.
- 6 Diez J, Laviades C, Mayor G, Gil MJ, Monreal I. Increased serum concentrations of procollagen peptides in essential hypertension. Relation to cardiac alterations. *Circulation* 1995;**91**:1450–6.
- 7 Matsumori A, Kawai C, Yamada T, Ohkusa T, Morishima S, Tamaki N, et al. Mechanism and significance of myocardial uptake of antimyosin antibody in myocarditis and cardiomyopathy: clinical and experimental studies. *Clin Immunol Immunopathol* 1993;**68**:215–9.
- 8 Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billingham ME, et al, and the Myocarditis Treatment Trial Investigators. A clinical trial of immunosuppressive therapy for myocarditis. *N Engl J Med* 1995;**333**:269–75.