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Serial Analysis of Troponin I Levels in Patients with Ischemic and Nonischemic Dilated Cardiomyopathy

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Summary

Background: Ongoing myocardial cell damage forms the basis for progression of chronic heart failure. Evidence is accumulating that progressive loss of cardiac myocytes is associated with the release of cardiac troponin I (cTnI).

Hypothesis: This study sought to determine whether levels of cTnI are of prognostic value for risk stratification of patients with chronic heart failure.

Methods: Release of cTnI was measured by conventional enzyme immunoassay following serum ultrafiltration in 58 consecutive patients hospitalized for chronic heart failure and 31 healthy volunteers serving as control group. Determination of serum levels was performed every 2 weeks over a time interval of 3 months. According to the results of coronary angiography, patients were divided into Group D showing normal coronary arteries (n = 33, ejection fraction 27 ± 6.1%) and Group I showing severe coronary heart disease (n = 25, ejection fraction 28.8 ± 7.8%). Survival of patients was evaluated after a mean time interval of 3 years.

Results: The mean cTnI serum level over all measurements was 0.66 ± 1.8 ng/ml in patients versus 0.11 ± 0.48 ng/ml in volunteers. At all six points of analysis, the mean cTnI serum level was significantly different ($p < 0.001$) between patients and volunteers. There was no significant difference between patients with and without coronary heart disease following hospital discharge, however, troponin release was significantly different between survivors and nonsurvivors (n = 27) (0.56 ng/ml vs. 0.84 ng/ml; $p < 0.05$).

Conclusion: Permanent cTnI release is a common finding in patients with chronic heart failure and a strong prognosticator. In this setting, coronary morphology seems to play a minor role for disease progression.

Key words: cardiac function, cardiovascular anatomy, cardiovascular biochemistry, cardiovascular pathology, clinical trials, heart failure, ischemic heart disease, troponin, trigger event

Introduction

While the incidence of many forms of cardiovascular disease, such as stroke or myocardial infarction (MI), have reached their maximum in Europe and the United States, the incidence of heart failure (HF) has markedly increased. Heart failure is associated with high rates of morbidity and mortality, especially in those patients presenting with an advanced state of the disease. Although the pharmacologic treatment of HF, including beta blockers and converting enzyme inhibitors as key drugs, is clearly defined, scant information exists regarding the underlying mechanism of disease progression. Following the trigger event, a variety of compensatory neurohumoral processes are activated, resulting in a normal cardiac output in the presence of elevated filling pressures, thus keeping the patient asymptomatic.[1–3] However, over time the patient will undergo transition from asymptomatic to symptomatic HF due to cardiac decompensation on the basis of left ventricular (LV) remodeling with subsequent myocardial damage. Up to now, the role of biochemical markers indicating severe progression of the disease has yet to be defined. In this regard, the analysis of cardiac troponin I (cTnI) serum levels in patients with chronic HF has attracted recent interest.[4] There is no doubt that on the cellular level HF is associated with progressive myocyte loss through necrotic cell death, most likely due to excessive neurohumoral stimulation.[5] This observation is confirmed by recent studies showing increased levels of cardiac cTnI in patients with advanced HF.[6, 7] Furthermore, evidence is accumulating that quantitative determination of cardiac cTnI may be of prognostic value, allowing risk stratification in diseased patients;[4, 7, 8] however, cTnI release may vary depending on the disease progression and the

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filling dynamics of the left ventricle. Serum levels of cTnI may be extremely low compared with those measured in acute coronary syndrome, and in some patients the levels may be below the limit of detection of conventional assays, requiring improved test sensitivity for analysis and repeated measurements. Therefore, the current study was designed to follow closely patients hospitalized for HF for 3 months. Serial measurements of cTnI release were performed every 2 weeks using both a conventional enzyme immunoassay and an analysis subsequent to an extra processing step of ultrafiltration. Following a time interval of 3 years, patients' survival was analyzed and compared with troponin release.

Patients and Methods

Patients hospitalized with clinical diagnosis of chronic systolic HF (> 3 months' duration on stable medical therapy) were candidates for the study. Patients with MI < 4 weeks before hospitalization, acute coronary syndromes, liver disease, renal failure, uncontrolled hypertension, or acute pulmonary edema were excluded. A total of 58 consecutive patients formed the final study population. All patients were in New York Heart Association (NYHA) class II–III; the time for hospitalization was 3–9 days, with a mean of 6 ± 3 days. Study patients received conventional pharmacologic therapy including beta blockers and angiotensin-converting enzyme (ACE) inhibitors; 25 patients had ischemic and 33 nonischemic cardiomyopathy. Following hospital discharge, patients were closely followed for 3 months, including physical examination every 2 weeks. Two patients with ischemic cardiomyopathy had to be excluded from further study participation due to severe cardiac events 6 and 8 weeks after study entrance, respectively. After a time interval of 3 years, survival of patients was reexamined by direct telephone contact.

Thirty-one healthy volunteers (14 women, 17 men; mean age 22 ± 6 years) served as controls. Their history was free of any cardiac and noncardiac disease. Patients and volunteers gave written informed consent before study inclusion.

Statistics

Patients' characteristics were compared with the unpaired *t*-test for quantitative data and with the chi-square test or Fisher's exact test for qualitative data. Comparison of cTnI serum levels was performed with the Wilcoxon test for paired data and the Mann and Whitney tests for unpaired data. General time dependency within each group was analyzed with Friedmann's test. The cTnI serum levels before and after ultrafiltration were compared with the paired *t*-test. All tests were performed two-sided on the level $\alpha = 0.05$.

Left Heart Catheterization

Coronary and LV angiography were performed in all study patients as part of their clinically mandated evaluation. The criteria for inclusion were LV ejection fraction (LVEF) < 35% and a diastolic volume index > 100 ml/m^2 body surface area (calculated from the LV angiogram in RAO-projection; Hicor®, Siemens Medical Solutions, Mountainview, Calif.).

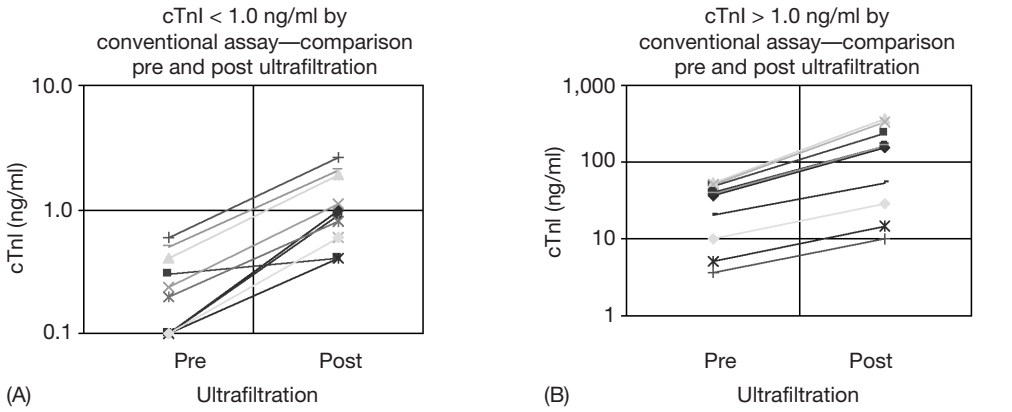
Coronary arteriography of the left and right coronary artery was performed in at least four and two projections, respectively. Coronary angiograms were reviewed by two independent observers experienced in angiographic interpretation. Ischemic cardiomyopathy was diagnosed in the presence of one coronary stenosis > 50% in at least one major coronary vessel, together with corresponding wall motion akinesia; in the presence of normal coronary arteries and global hypokinesia, the diagnosis of nonischemic cardiomyopathy was established.

Table I summarizes the clinically relevant data for the patients. Hemodynamic parameters of LV function were compa-

TABLE I Characteristics of patients with heart failure

	Cardiomyopathy		
Finding	Ischemic (n = 25)	Nonischemic (n = 33)	p Value
Age, years	67 ± 10	58 ± 12	0.002
Sex, female/male	7/18	9/24	0.84
NYHA class (%)			0.65
NYHA II	64	70	
NYHA III	36	30	
Arterial hypertension (%)	80	73	0.52
Diabetes mellitus (%)	36	30	0.40
Hyperlipidemia (%)	88	36	< 0.001
Tobacco (%)	36	42	0.62
Alcohol abuse (%)	12	52	< 0.002
Medication at study entrance (%)			
Digitalis	52	61	0.51
Beta blockers	40	12	0.014
ACE inhibitors	84	88	0.72
Diuretics	100	91	0.25
ECG (%)			
Atrial fibrillation/-flutter	52	36	0.23
Bundle-branch block	32	45	0.30
Angiography			
Coronary artery disease			Not tested
1-vessel (%)	36	0	
2-vessel (%)	28	0	
3-vessel (%)	36	0	
LVEF (%)	29 ± 8	28 ± 8	0.64
Mitral insufficiency grade III or IV (%)	52	39	0.34
End-diastolic volume index (ml/m^2)	105 ± 32	110 ± 29	0.54
End-systolic volume index (ml/m^2)	74 ± 28	72 ± 27	0.78
LVEDP (mmHg)	20.3 ± 8.2	18.8 ± 5.8	0.28

Abbreviations: NYHA = New York Heart Association, ACE = angiotensin-converting enzyme, ECG = electrocardiogram, LVEF = left ventricular ejection fraction, LVEDP = left ventricular end-diastolic pressure.



Total	1	2	3	4	5	6	7	8	9	10
Pre UF	0.1	0.3	0.4	0	0	0	0.6	0	0.5	0.1
Post UF	1.0	0.4	1.9	0.6	0.4	0.9	2.6	0.6	2.1	0.6

Total	1	2	3	4	5	6	7	8	9	10
Pre UF	37	49	53	50	5.2	41	3.7	21	38	10
Post UF	152	232	371	328	15	160	10	53	165	29

FIG. 1 Effect of ultrafiltration (UF) on cardiac troponin I (cTnI) concentration. (A) Low concentration range (< 1.0 ng/ml); (B) high concentration range (≥ 1.0 ng/ml).

rable in both groups, in contrast to severe coronary artery sclerosis which was exclusively present in patients with ischemic cardiomyopathy.

Determination of Serum Cardiac Troponin I Levels

For analysis of serum cTnI levels, a conventional enzyme-immunoassay, used world-wide, was utilized. The cTnI levels are reported in ng/ml; the lowest measurable concentration with this assay is 0.1 ng/ml (Abbott, AxSYM).

Before quantitative measurements, serum ultrafiltration was performed using a conventional filtration unit (Ultrafree®-4, Millipore Corp., Bedford, Mass., USA). This extra step was used to enrich cTnI concentration. The active surface of the membrane was 2 cm^2, the molecular weight limit for separation was chosen to be 5,000 dalton, time of centrifugation was 45 min (5,000 U/min, Sigma Laboratory Centrifuges GmbH, Osterode am Harz, Germany). Increase in cTnI concentration was proportional to the reduction of volume; 2.0 ml was introduced into the filtration unit resulting in a final sample volume of about 0.40 ml. Figure 1 shows the serum cTnI levels before and after ultrafiltration (UF). Ultrafiltration led to a significant concentration of cTnI (0.2 ± 0.2 ng/ml → 1.1 ± 0.8 ng/ml, n = 10, p = 0.001; 30.8 ± 19.2 ng/ml → 151.5 ± 128.8 ng/ml, n = 10, p = 0.007).

Table II shows the reproducibility of the measurements. Repeated analysis of cTnI was performed in one patient's serum. Each sample underwent UF before analysis.

Determination of cTnI levels was serially performed in all patients. Blood samples were taken every 2 weeks, starting at the time of hospitalization and continuing after discharge for 3 months. All cTnI levels presented in the result section were obtained following serum ultrafiltration.

Results

Cardiac Troponin I Serum Levels Following Ultrafiltration

Figure 2 shows the median serum cTnI levels in both patient groups compared with volunteers. The median of "0" means ≥ 50% of the patients have the cTnI plasma level "0." There were significant differences in the cTnI serum levels at all time

TABLE II Reproducibility of cardiac troponin I measurements

Sample	Measurement 1	2	3	4	5	6	7	8	Mean ± SD	n	Precision (SD/mean)
1	1.4 [a]	1.4	1.4	2.2	1.5	1.2	1.4		1.5 ± 0.3	7	0.21
2	1.1	0.7	0.7	0.9	0.7	0.6	0.6	0.7	0.8 ± 0.2	8	0.23
3	0.1	0.2	0.3	0.2	0.8	0.4	0.3	0.2	0.3 ± 0.2	8	0.69
4	0.8	0.7	0.9	0.6	0.9	0.9	0.6		0.8 ± 0.1	7	0.18
5	0	0	0	0	0	0	0	0	0 ± 0	8	

[a] ng/ml.

Abbreviation: SD = standard deviation.

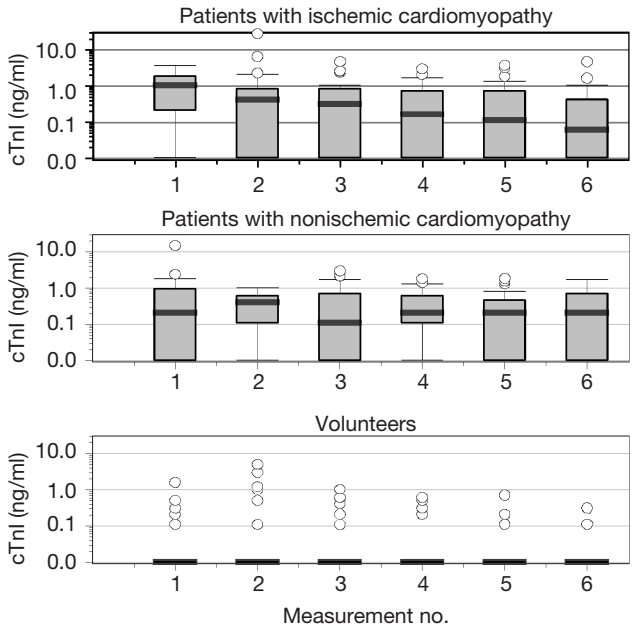


FIG. 2 Comparison (boxplot) of median serum levels in both patient groups and volunteers over 12 weeks. Measurements were performed every 2 weeks. CTnI = cardiac troponin I.

points of analysis between both patient groups and volunteers ($p < 0.007$ each). Ischemic and nonischemic patients differed at the first measurement only ($p = 0.011$). The cTnI level steadily decreased in patients with ischemic cardiomyopathy while there was no corresponding tendency in patients with nonischemic heart failure; in both patient groups the cTnI remained elevated during the whole study period.

Table III compares the serum troponin levels in all patients with those obtained in volunteers. Differences were significant at all time points of analysis.

Table IV and Figure 3 showed a comparison of troponin release (mean values and median) in survivors and nonsurvivors. Troponin release was significantly different between surviving and nonsurviving patients with chronic HF (reexamination after 3 years interval). This was true for both patients with ischemic and nonischemic cardiomyopathy, respectively.

Discussion

Troponin I is a regulatory subunit of the troponin complex associated with the actin thin filament within muscle cells. There are two selected isoforms of cTnI, which are structurally distinct although similar in molecular weight (19.000 daltons). The cardiac form of cTnI contains 31 additional amino acids at the N-terminus, resulting in a molecular weight approaching 24.000 daltons.[9]

The role of cTnI analysis in MI and acute coronary syndrome is clearly defined. However, in chronic HF—especially with respect to its etiology—clinical value has not yet been clarified.[4, 10, 11] The progressive character of the disease is well known and most likely due to progressive myocardial cell damage. The process of ongoing minor myocardial injury may be associated with some form of cTnI release, which might be detectable by serial analysis of cTnI. This hypothesis is confirmed by a previous study showing that patients with progressive HF had elevated cTnI serum levels; however, cTnI concentrations were extremely low and therefore difficult to detect when conventional tests were used.[8]

The current study differs from those mentioned above with respect to a consequent follow-up of patients after hospital discharge, including cTnI analysis in a serial manner, taking into account a possibly intermittent release of cTnI. Furthermore, it allows a clear separation of patients regarding the trigger event, thus possibly defining its role for disease progression.

Cardiac Troponin I Release in Patients with Heart Failure

A certain amount of cTnI release was observed during the whole study interval and was comparable in both patient groups after hospital discharge. The hemodynamic overloading may be most pronounced in patients in whom chronic cardiac failure has led to severe cardiac decompensation, giving a possible explanation for the fact that cTnI serum levels were generally found to be higher at the time of hospitalization (Table III). In this setting, the maximum of cTnI serum levels was found in patients with ischemic cardiomyopathy within the first weeks after study entrance, suggesting that reduction in oxygen supply due to severe coronary artery disease had enhanced the complex process of myocardial cell damage in the chronic failing heart. In the ischemic group cTnI continuously

TABLE III Troponin serum levels in patients and volunteers

	1	2	3	4	5	6	Mean	p Value
Patients (n)	57 [a]	58	58	57 [a]	55 [a]	52 [a]		
cTnI (ng/ml) mean + SD	1.0 ± 2.1	1.0 ± 3.6	0.6 ± 0.9	0.5 ± 0.6	0.5 ± 0.8	0.4 ± 0.7	0.66 ± 1.8	
Median	0.40	0.40	0.25	0.20	0.20	0.10		0.126
Volunteers (n)	31	31	31	31	27	21		
cTnI (ng/ml) mean+SD	0.1 ± 0.3	0.3 ± 1.0	0.08 ± 0.2	0.06 ± 0.15	0.04 ± 0.14	0.02 ± 0.07	0.11 ± 0.48	
Median	0.00	0.00	0.00	0.00	0.00	0.00		0.400
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

[a] Analysis not possible in one or more sample volumes.

TABLE IV Troponin serum levels (cTnI, ng/ml) of survivors and nonsurvivors

Survival	n	cTnI (ng/ml) Mean	cTnI (ng/ml) Median
Ischemic cardiomyopathy			
Survivors	11	0.89 ± 1.3	0.53
Nonsurvivors	12	1.07 ± 1.1 [a]	0.51
Nonischemic cardiomyopathy			
Survivors	14	0.29 ± 0.17	0.25
Nonsurvivors	15	0.62 ± 0.56 [a]	0.56 [a]
Total			
Survivors	25	0.56 ± 0.93	0.27
Nonsurvivors	27	0.84 ± 0.91 [a]	0.54 [a]

[a] $p < 0.05$.

decreased; however, the total amount of cTnI still was higher in both survivors and nonsurvivors, respectively, compared with the nonischemic group (Table IV). After cardiac recompensation and hospital discharge, persistent cTnI release that was comparable in both groups indicates continuing progression of HF on the cellular level despite intensive pharmacologic therapy. After a 3-year time interval, more than 50% of the patients died. Both death rate and troponin release were independent of coronary anatomy, suggesting minor effects of coronary heart disease for the disease progression.

Cardiac Troponin I Release in Healthy Volunteers

In agreement with previous work, the current study demonstrated cTnI release in some healthy volunteers; however, in contrast to patients with HF, cTnI detection was exceptional.[10, 12] Based on the data published so far, a clear statement about this phenomenon is not possible. In our opinion, cTnI release most likely reflects normal turnover of myocytes under physiologic conditions.

Role of Trigger Event

An initial decline in the pumping capacity of the heart represents the first step in the pathogenesis of HF. Regardless of the nature of the trigger event, a variety of compensatory processes are activated. These include activation of the neurohumoral system, a key mechanism resulting in the ability of the heart muscle to restore cardiovascular function which thus causes the patient to be preliminarily asymptomatic.[13]

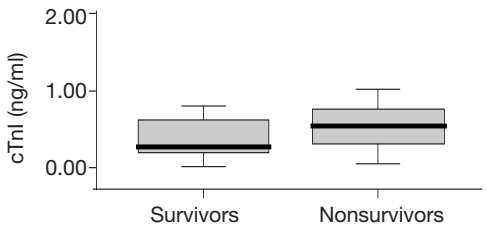


FIG. 3 Comparison of median troponin serum levels in surviving (n = 25) and nonsurviving (n = 27) patients with either ischemic or nonischemic cardiomyopathy. cTnI = cardiac troponin I.

Conclusion

The current study allows comparison of two patient groups that were similar with regard to severity of LV dysfunction and volume overload but different concerning the trigger event. Despite this difference, cTnI release and its impact on survival was similar between these groups following hospital discharge, suggesting a minor influence of the trigger event on disease progression. These data also confirm the generally accepted hypothesis that compensatory mechanisms such as activation of the adrenergic nervous system, the renin-angiotensin system, and the cytokine system mainly contribute to disease progression.[14–17]

Study Limitations and Clinical Implications

It is possible that myocardial ischemia due to significant coronary heart disease enhances cTnI release in patients with ischemic cardiomyopathy. However, as cTnI release was comparable in both our patient groups after hospital discharge, it appears that mechanisms other than decreased myocardial oxygen supply due to epicardial coronary stenosis are responsible for the cTnI release.

In agreement with the findings of other groups,[11] cTnI serum levels were extremely low compared with those obtained in acute coronary syndromes or MI. Therefore, the assay was operated in a low concentration range with the implications of a certain inaccuracy of measurement. However, all three groups in this study were affected to the same degree, suggesting minor effects on the comparison among groups.

Ventricular decompensation in chronic HF is assumed to be associated with increase in troponin release. Combining cTnI and natriuretic peptide analysis, which was not performed in this study, may offer a new risk-stratifying tool that detects cellular injury as well as ventricular overload.[18]

The healthy volunteers are significantly younger than the mean age of the patients. Thus, it cannot be totally ruled out that detection of cTnI in patients is associated with just older age; however, this assumption is not in agreement with the current literature and neglects the role of cTnI as an age-independent prognosticator in HF.[11]

Determination of creatinine clearance was not performed. Therefore, one might assume that cTnI levels reflect changes in renal function rather than ongoing myocardial injury; however, cTnI levels were shown to be independent of renal function.[19]

In view of a new test generation using a chemoluminescence technique, the clinical value of ultrafiltration has to be newly defined. This new test generation is now commercially available and improves test sensitivity significantly.

Determining optimal pharmacologic treatment may be markedly facilitated using cTnI monitoring. Ultrasensitive quantification will better allow the definition of particular subgroups of patients at high risk for disease progression, special therapeutic interventions, and adverse outcomes.

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