# Release characteristics of cardiac proteins after reversible or irreversible myocardial damage

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# Chapter 5

## Serum troponin concentrations in patients with congestive heart failure are stretch-dependent

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## Abstract

**Purpose:** Although serum cardiac troponins are specific biomarkers of irreversible cell damage, serum levels of cardiac troponins are frequently elevated in pathological conditions in which myocardial necrosis is not a prominent aspect. Recently our group has reported that cardiomyocytes *in vitro* release cardiac troponin I (cTnI) in the absence of necrosis by a stretch-related mechanism, mediated by integrin stimulation. We hypothesize that patients with congestive heart failure (CHF) with elevated serum cTnT levels at baseline had myocardial cTnT release due to myocardial stretch in cardiac overload, instead of cTnT release due to necrosis.

**Methods:** To test this hypothesis we selected 11 patients with CHF who had elevated serum cTnT levels at baseline (the CHF⊕ group), 11 patients with CHF who had serum cTnT levels at baseline <0.01 µg/L (the CHFΘ group) and 12 patients with AMI and elevated cTnT serum levels (AMI group). Serum levels of cTnT, lactate dehydrogenase (LDH) and amino-terminal propeptide of brain natriuretic peptide (NT-proBNP) were assayed.

**Results:** The CHF⊕ had ≈ 4-fold higher serum NT-proBNP levels at baseline than the CHFΘ group, indicating higher overload in CHF⊕ patients than in CHFΘ patients ($p<0.001$). Serum LDH activity did not differ between CHF⊕ and CHFΘ. Serum cTnT/LDH ratios in CHF⊕ and CHFΘ were 0.510±0.186 ng/U and 0.035±0.0029 ng/U, respectively ($p<0.001$), indicating preferential cTnT release from hearts of CHF⊕ patients.

**Conclusion:** These results support our experimental findings with integrin-stimulated cardiomyocytes *in vitro*. Apparently, also in patients with CHF preferential cTnT release may be the result cardiac overload and associated stretch, instead of myocardial necrosis.



## Keywords

congestive heart failure, acute myocardial infarction, cardiac troponin, necrosis, myocardial stretch

# Introduction

In 2000 the joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction published a consensus document about the redefinition of myocardial infarction[1]. In this document the presence of a serum troponin concentration exceeding the95$^{th}$ or 99$^{th}$ percentile of the reference distribution is considered to confirm the diagnosis "myocardial infarction", even in case of microscopic zones of myocardial necrosis. Since than, many reports have been published that make the standpoint of the joint Committee highly disputable. Renal failure causes an increase of serum troponin levels due to an impaired renal clearance of troponin and troponin degradation products, in the absence of any cardiac pathology[2]. Also pulmonary embolism, cerebrovascular accident, diabetic ketoacidosis, exacerbation of chronic obstructive pulmonary disease, gastrointestinal bleeding and liver cirrhosis can be associated with abnormally high serum troponin levels without an evident role for cardiac pathology[3-5]. In conditions like congestive heart failure, myocarditis, unstable angina pectoris, doxorubicin toxicity as well as in athletes after ultra-endurance exercise an elevated troponin concentration in serum has been reported frequently[6-11]. Irreversible myocardial injury is not a prominent aspect under these conditions; however, the presence of myocardial necrosis cannot be excluded as it is extremely difficult to prove the absence of microscopic zone of necrosis.

In the past, it has been demonstrated that vital ventricular cardiomyocytes may release proteins upon specific stimuli. To this group of proteins belong atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) released from cardiomyocytes during treatment with endothelin-1[12], angiotensinII released from stretched cardiomyocytes in vitro[13], basic fibroblast growth factor (FGF2) from cardiomyocytes subjected to increased mechanical activity[14], vascular endothelial growth factor (VEGF) from cardiomyocytes upon pulsatile stretch[15], transforming growth factor-$\beta_1$ (TGF-$\beta_1$) from cardiomyocytes isolated from pressure overloaded hearts, as well as from cardiomyocytes stimulated by norepinephrine in vitro[16]. Cell stretch is perceived by sarcolemmal integrins that couple extracellular matrix proteins to the intracellular cytoskeleton[17]. Matrix ligands that engage with integrins contain certain domains, one of which is the RGD domain present in fibronectin[18].

Recently our group has reported that rat ventricular cardiomyocytes *in vitro* release cardiac troponin I (cTnI) upon integrin stimulation by RGD[19]. This cTnI release is not accompanied by the release of lactate dehydrogenase (LDH), indicating that cTnI release due to integrin stimulation differs from cTnI release from rat ventricular cardiomyocytes that were subjected to necrosis by metabolic inhibition *in vitro*[20;21]. In the latter case, LDH release and cTnI release occur both. Stretch and integrin stimulation with a RGD-containing peptide seem to open a release port for certain proteins that is not open for LDH. Whether this release port is identical to the transient increase in sarcolemmal permeability upon cardiac overload, cardiomyocyte stretching and increased mechanical activity of isolated cardiomyocytes is not clear yet[22]

In the present study we have investigated whether we could find this phenomenon in the human situation. We selected two groups of patients with congestive heart failure (CHF), those with and without elevated serum cardiac troponin-T (cTnT) levels at baseline. Our working hypothesis is that release of cTnT from failing hearts is not necessarily the result of ongoing necrosis, but could also be the result of cell stretch leading to integrin stimulation. As a representation of patients with myocardial necrosis, we included a third group consisting of 12 patients with acute myocardial infarction (AMI).

## Materials and Methods

### Patients

This study includes three groups of patients: (1) 11 patients with congestive heart failure who had baseline serum cTnT levels >0.01 µg/L (indicated by CHF⊕), (2) 11 patients with congestive heart failure who had baseline serum cTnT levels <0.01 µg/L (indicated by CHFΘ), and (3) 12 patients with acute myocardial infarction, all having serum cTnT levels >0.01 µg/L (indicated by AMI). CHF patients had moderate-to-severe heart failure (NHYA class III-IV), LV ejection fraction ≤35%, and QRS duration >120 ms. Patients with CHF and a recent myocardial infarction (< 3 months) or decompensated heart failure were excluded. Patients with AMI had been treated successfully by percutanous coronary intervention. All patients gave informed consent to participate in this study.

### Biochemical analysis

At baseline blood samples were obtained, and serum samples were stored at -80°C. Serum levels of NT-proBNP were determined using an automated immunoassay (Elecsys, Roche). The reference range was 0-400 pg/mL, and the intra-assay variability was 1.8% at high concentrations of NT-proBNP (800 pg/mL), and 2.7% at low concentrations (2.1 pg/mL).

Serum cardiac troponin T (cTnT) concentrations were determined by Roche assay (Elecsys, Roche). The reference value is <0.01 µg/L, and the inter-assay variability was 8% and 7% at concentrations of 0.134 µg/L and 2.85 µg/L, respectively.

Lactate dehydrogenase (LDH) activity in serum samples was measured spectophotometrically according to Wroblewski & LaDue[23].

### Statistical analysis

Data were expressed as mean ± SD and compared with the two-tailed Student's t-test for unpaired data. A p-value < 0.05 was considered statistically significant.

## Results

### NT-proBNP

Patients in the CHF⊕ group had considerably higher serum NT-proBNP concentrations at baseline than patients in the CHFΘ group (4036±800 ng/L vs. 921±189 ng/L, p<0.001), and than patients in the AMI group (1754±446 ng/L, p<0.05) (Table).

**Table**. Serum levels of NT-proBNP, cTnT and LDH at baseline in the three groups of patients included in the study. Indicated are mean ± SEM.

| | CHF⊕ group | CHFΘ group | P* | AMI group | P# |
|---|---|---|---|---|---|
| n | 11 | 11 | | 12 | |
| NT-proBNP (ng/L) | 4036±800 | 921±189 | <0.001 | 1754±446 | <0.05 |
| cTnT (µg/L) | 0.074±0.027 | <0.01 | <0.001$ | 4.14±1.29 | <0.001 |
| LDH (U/L) | 154±10.2 | 152±13.6 | n.s. | 534±149 | <0.001 |
| cTnT/LDH (ng/U) | 0.510±0.186 | 0.035±0.003 | <0.001 | 6.90±1.42 | <0.001 |

* t-test CHF⊕ group versus CHFΘ group, # t-test AMI group versus CHF⊕ group, $ by selection.

Considering the serum NT-proBNP concentration as a general indicator of cardiac overload, we may conclude that a CHF patient having a serum cTnI level of > 0.01 µg/L at baseline has on average ≈ 4-fold higher cardiac overload than a CHF patient with a serum cTnT level of <0.01 µg/L at baseline. Therefore we expected that in the CHF⊕ group serum cTnT and NT-proBNP levels are correlated, but the correlation was not significant (p=0.40, n.s.) (Fig. 1a). In the AMI group the serum cTnT and serum NT-proBNP levels are correlated significantly (r= 0.77, p<0.01.) (Fig 1b).

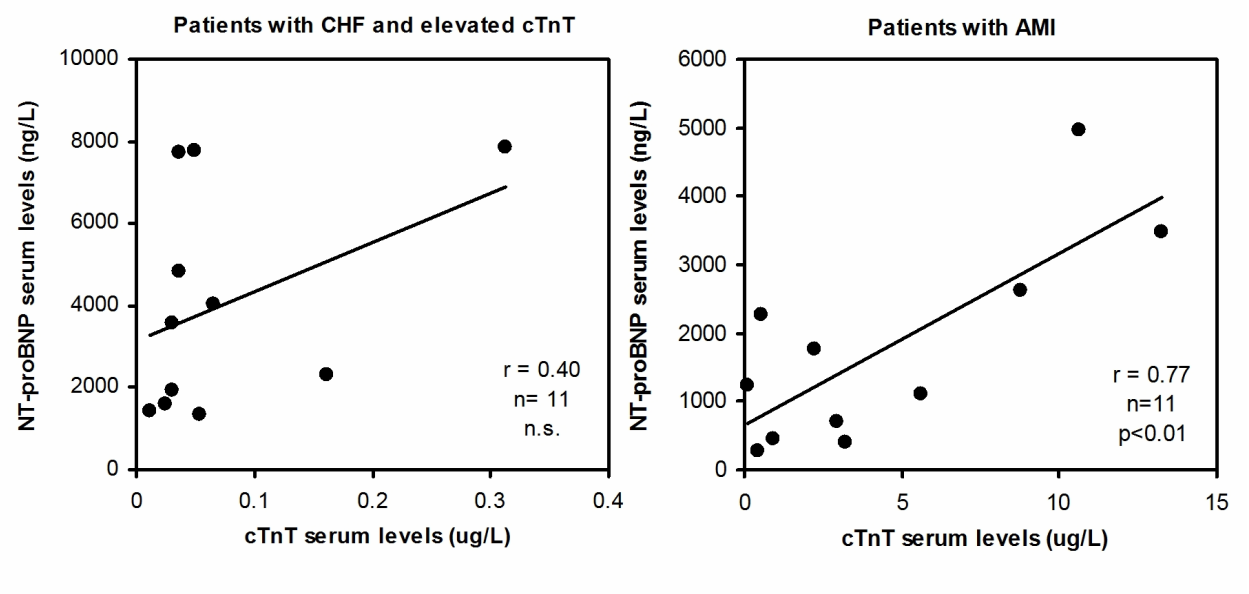


**Figure 1.** Plots of serum NT-proBNP levels at baseline versus serum cTnT at baseline in (**a**) CHF⊕ patients and (**b**) patients with AMI.

## cTnT-LDH relationship

A higher cardiac overload in the CHF⊕ group, compared to the CHFΘ group, may indicate more stretch of ventricular myocardium, leading to cTnT release without concomitant LDH release. The latter phenomenon we have observed in neonatal rat ventricular cardiomyocytes in which integrins were stimulated by RGD-containing pentapeptide.

Therefore we expected a higher ratio of serum cTnT and LDH in the CHF⊕ group that in the CHFΘ group. This turned out to be the case: cTnT/LDH ratio of the CHF⊕ group was 0.510±0.186 ng/U and 0.035±0.003 ng/U in the CHFΘ group (p<0.001), a ≈ 15-fold

difference. Secondly, the average LDH activity in serum was 154±10 U/L and 152±13 U/L in CHF⊕ and CHFΘ group respectively (n.s.) (Table). In the CHF⊕ group serum LDH and cTnT levels were not correlated (r=-0.14, n.s.) (Fig. 2a), whereas in AMI patients serum LDH and cTnT levels are correlated highly significantly (r=0.908, p<0.001) (Fig. 2b).

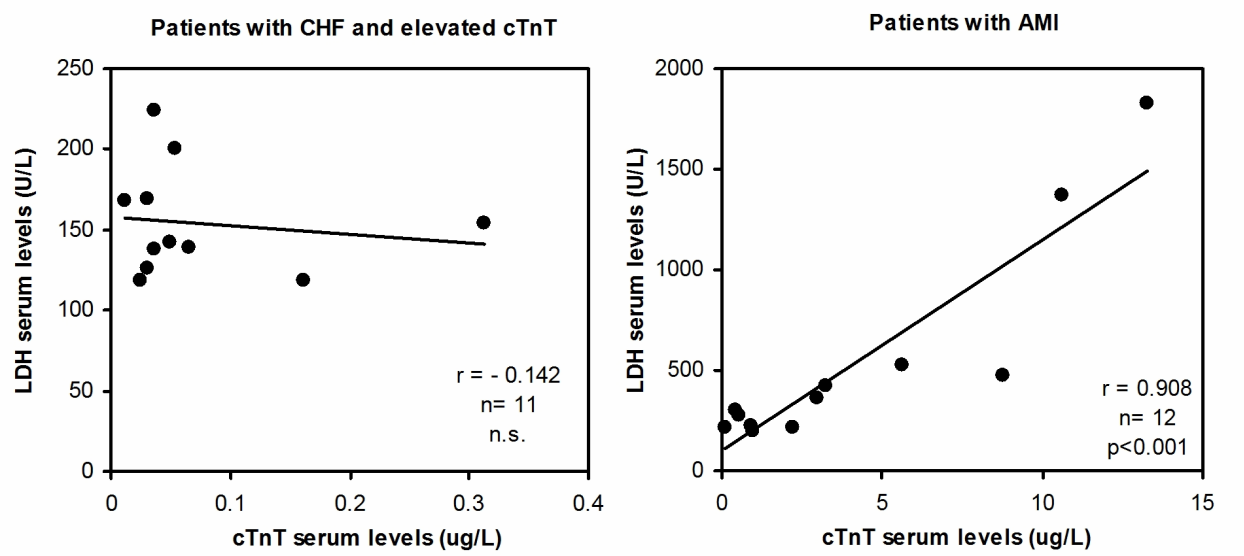


**Figure 2.** Plots of serum LDH activity at baseline versus serum cTnT at baseline in (**a**) CHF⊕ patients and (**b**) patients with AMI.

# Discussion

The current study presents results that support our experimental finding observed in neonatal rat ventricular cardiomyocytes showing preferential cTnI release upon integrin stimulation by a RGD-containg pentapeptide as a model of myocardial stretch in cardiac overload[19]. We hypothesized that CHF patients with elevated serum cTnT at baseline had myocardial cTnT release due to myocardial stretch in cardiac overload, instead of myocardial cTnI release due to necrosis. Indeed we observed a ≈ 4-fold higher serum NT-proBNP level at baseline in the CHF⊕ group than in the CHFΘ group, interpreted by us as a higher cardiac overload and associated myocardial stretch in the CHF⊕ group than in the CHFΘ group.

Both CHF⊕ and CHFΘ groups had similar serum LDH activity, whereas in patients with AMI serum LDH activity was on average ≈ 2-fold higher than in the CHF⊕ and CHFΘ groups. An increased ratio of serum cTnT/LDH in the CHF⊕ group indicates a preferential release of cTnT without release of LDH, like we found in rat cardiomyocytes subjected to integrin stimulation in vitro[19]. In the CHF⊕ group the serum cTnT/LDH ratio was on average ≈ 15-fold higher that in the CHFΘ group, confirming the experimental results.

The transient increase of serum membrane permeability, induced by mechanical forces that impose shear, tensile and compressive stresses on muscle cells in particular, has been considered responsible for the release of proteins like ANP, BNP, bFGF, AngII, VEGF and TGF-$\beta_1$ from cardiomyocytes challenged with mechanical stress. These serum membrane disruptions have been termed “cell wounding” and are considered to last for seconds only to avoid cell death. Serum membrane disruptions are repaired by a resealing mechanism that requires $Ca^{2+}$-influx and a high rate of $Ca^{2+}$-regulated exocytosis. It appears that $Ca^{2+}$-dependent mechanisms for cell membrane resealing may involve vesicle delivery, docking, and fusion, very similar to the exocytosis of neurotransmitters[24]. The released proteins are rapidly replenished by stress and shear stress-induced upregulation of gene expression.
To date, it is not clear how preferential release of specific proteins is regulated. Why is cTnI release from integrin stimulated cardiomyocytes and cTnT release from CHF⊕ patients not accompanied by LDH release? Future investigations of vesicular protein contents should elucidate the mechanism of preferential protein release from stressed and stretched cardiomyocytes.

**Conclusions**

In patients with CHF preferential cTnT release may be the result of cardiac overload and associated stretch, instead of myocardial necrosis. These results support our experimental findings with integrin-stimulated cardiomyocytes *in vitro*.

### Limitations

In this study, the number of patients was relatively small and a larger population is needed to confirm our findings.

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