

Cardiac troponin T degradation after acute myocardial infarction

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The heart muscle specific isoform of troponin T (cardiac troponin T, cTnT) is released after acute myocardial infarction (AMI) and shows a characteristic biphasic change in the serum time concentration curve (1). In patients with early reperfusion (>3.5 hours), a rapid initial rise of cTnT is followed by a delayed second release. This is probably the result of the fast release of the unbound cytosolic fraction (6%), followed by a more slow release of the cTnT fraction bound to the myofibrils (94%) (1). The Roche immunoassay for cTnT, without cross-reactivity to the skeletal isoforms, has become widely used and troponin (both T and I) is now the standard in the laboratory diagnosis of acute coronary syndromes (2).

In addition to our reports (3, 4), there have been two reports by other groups describing both intact cTnT and its fragments in serum of patients with AMI. The first study used serum samples from three patients and used gel filtration (5). The second study detected one major (≈ 26 kDa) and two minor degradation products with Western blot in serum from one patient (6). The latter clearly showed a time related difference in the appearance of cTnT fragments. The first, however, did not. In the present study we thoroughly investigated cTnT degradation at different time points after AMI.

Methods

Patients and Analytical methods

Serum samples sent to our laboratory for routine analysis were collected from 20 patients diagnosed with AMI. Routine analyses, including cTnT, CK-MB_{mass} and creatinine were performed within 1 hour after blood collection. cTnT (3rd generation) and CK-MB_{mass} were measured on an Elecsys 2010 analyzer (Roche Diagnostics) and creatinine on a Synchron LX20 analyzer (Beckman Coulter). Samples were stored at -20 °C until used for analysis.

Western Blotting

Serum samples were thawed and cTnT was purified according to the method we described earlier (4). In brief, samples were prepared for SDS-PAGE by adding 20 μ L of sample buffer to 80 μ L of purified cTnT sample. We applied 20 μ L of this mixture to a 4-15% linear gradient Tris-HCl polyacrylamide pre-cast gel. The Precision Plus Protein Standard was

used as the molecular mass marker. After electrophoresis, the gel was blotted onto a 0.45 μ m nitrocellulose membrane. The primary anti-cTnT antibody (4C5) was added at a 1:1000 dilution in wash buffer (PBS containing 0.2 mL/L Tween-20 and 6.7 g/L nonfat dry milk) and incubated overnight at 4 °C. The secondary peroxidase labeled goat-anti-mouse antibody was added at a 1:5000 dilution in wash buffer and incubated for 60 minutes at 4 °C. The membrane was developed using chemiluminescence and captured on Kodak X-Omat Blue film.

Statistical analysis

Statistical analysis was done with SPSS 11. The overall differences between the time points after AMI were tested with the non-parametric Kruskal-Wallis test. Because there was a significant difference ($p < 0.01$), the non-parametric Wilcoxon signed rank test was used for analysis of differences in the number of fragments and their average molecular weight at different time points after AMI. Bonferroni correction was used to adjust for multiple testing.

Results

All patients had rapid reperfusion (< 4 hours), either by thrombolysis, PTCA, PCI with stenting of one or more coronary arteries or rescue CABG. Two patients died during hospitalization at 14 and 20 days after onset of symptoms. Release kinetics for cTnT showed a characteristic biphasic change in the serum time concentration curve (Figure 1). The curve was then divided as shown in the inset of Figure 1A, where point 0 represents the last measurement of cTnT that was below the 99th percentile reference limit of <0.010 μ g/L. Point 2 indicates the highest cTnT measurement. Point 4 represents the second cTnT peak, and points 1, 3 and 5 samples in between. The mean time at points 0 – 5 was 0, 10, 13, 46, 101 and 198 hours.

Two researchers, blinded for cTnT, CK-MB_{mass} and creatinine levels, independently counted the number of cTnT fragments per sample and calculated their molecular weights based on the molecular mass markers (Figures 2 A and 2B). Intact cTnT (37 kDa) was detected in 15 patients (75%), however, only within the first 13 hours after AMI. In later samples, after the cTnT peak concentration at 13 hours, no intact cTnT could be detected and only cTnT fragments were identified. Evidence for the presence of the troponin TIC complex (± 79 kDa) was not found in any of these patients. During the first 13 hours, the number of cTnT fragments increased rapidly to 5,

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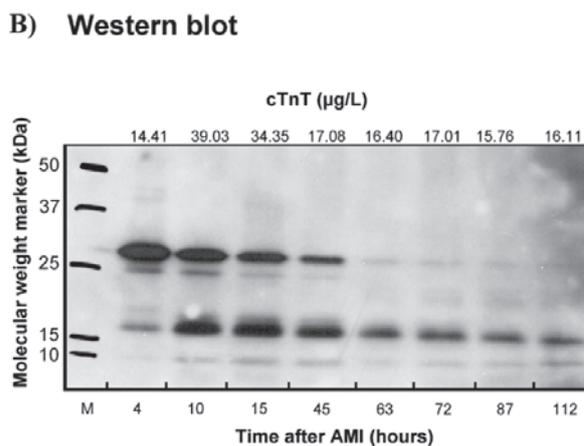
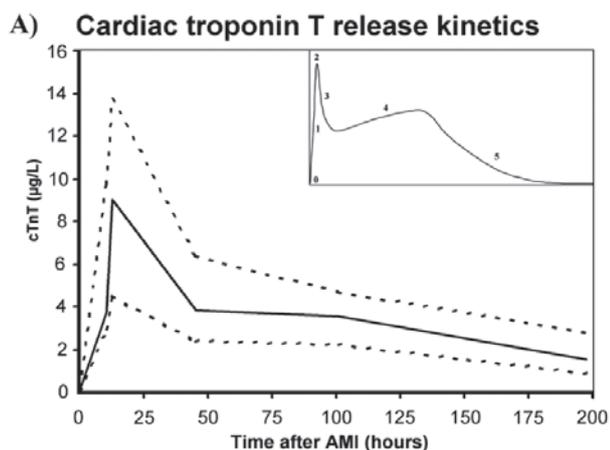


Figure 1. Release kinetics of cTnT, fragments and molecular weight. A) Typical biphasic cTnT release kinetics after AMI for 20 patients. The serum cTnT concentrations are represented as median and interquartile range (25 to 75%). B) Example of a Western blot from one patient analyzed in time.

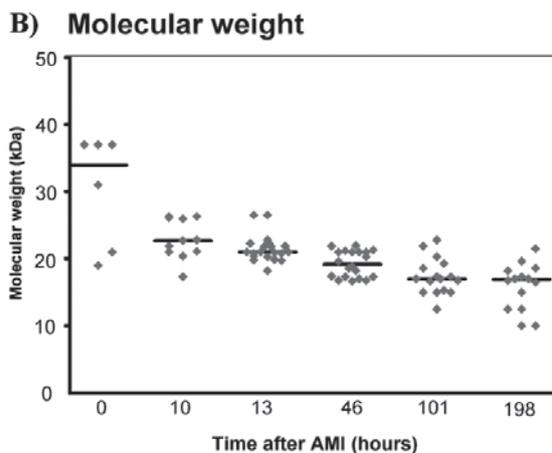
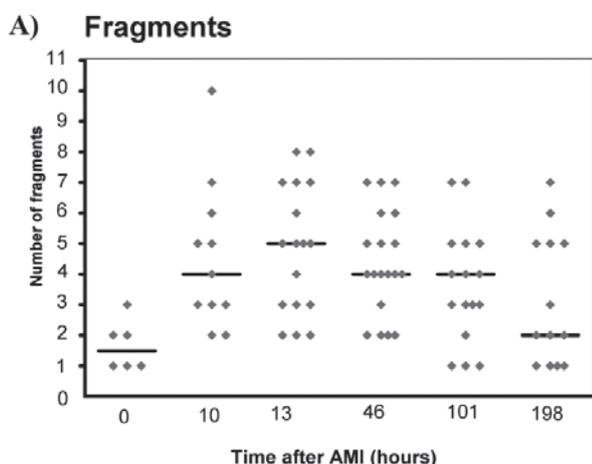


Figure 2. cTnT fragments and molecular weight in time. A) The number of cTnT fragments increased rapidly in the first 13 hours and then decreased slowly; immunoreactive fragments were still present in the circulation at 198 hours. B) The average molecular weight of all fragments showed intact cTnT to be rapidly degraded into smaller fragments. Horizontal lines represent median values, $n = 6, 11, 20, 20, 17$ and 14 at $0, 10, 13, 46, 101$ and 198 hours, respectively.

with a 25 kDa fragment being the most prominent. Thereafter, the number of fragments decreased slowly to 2 at 198 hours ($p=0.066$). The average molecular weight of all detected fragments was highest directly after AMI (30.3 kDa). From the peak cTnT at 13 hours, the average molecular weight (21.5 kDa) decreased significantly to 19.3 kDa at 46 hours ($p=0.006$), 17.2 at 101 hours ($p=0.006$) and 15.9 kDa at 198 hours ($p<0.012$).

Conclusions

Intact cTnT (37 kDa) was detected only during the first 13 hours of fast cTnT release. During the slow cTnT release phase, only smaller molecular weight immunoreactive fragments could be detected and no intact cTnT. With our study, we provide valuable data about the presence of intact cTnT in the early hours after onset of symptoms and the presence of immunoreactive fragments in serum of patients with AMI. Although cTnT has proven to be a valuable marker for acute coronary syndromes (2), its clearance and metabolism remain unclear and require further investigation. However, the results from our study indicate that fragmentation of cTnT is not likely to influence clinical interpretation of elevated cTnT levels.

References

1. Katus HA, Remppis A, Looser S, Hallermeier K, Scheffold T, Kubler W. Enzyme linked immuno assay of cardiac troponin T for the detection of acute myocardial infarction in patients. *J Mol Cell Cardiol* 1989; 21: 1349-1353.
2. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000; 36: 959-969.
3. Diris JH, Hackeng CM, Kooman JP, Pinto YM, Hermens WT, Van Dieijen-Visser MP. Impaired Renal Clearance Explains Elevated Troponin T Fragments in Hemodialysis Patients. *Circulation* 2004; 109: 23-25.
4. Michielsen EC, Diris JH, Hackeng CM, Wodzig WK, Van Dieijen-Visser MP. Highly sensitive immunoprecipitation method for extracting and concentrating low-abundance proteins from human serum. *Clin Chem* 2005; 51: 222-224.
5. Wu AH, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. *Clin Chem* 1998; 44: 1198-1208.
6. Labugger R, Organ L, Collier C, Atar D, Van Eyk JE. Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. *Circulation* 2000; 102: 1221-1226.