

# Full-size human cardiac troponin T and its fragments in the plasma of patients with acute myocardial infarction

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## Introduction

The measurement of cardiac troponin T (cTnT) in the blood is one of the most reliable methods of acute myocardial infarction (AMI) diagnosis. Whilst cTnT is known to be susceptible to the proteolytic degradation, there is still no consistent data describing the level and sites of degradation of cTnT molecules that are present in the blood of AMI patients. Meanwhile, degradation might have a significant influence on the precise immunodetection of cTnT. In this study, we aimed to determine the cTnT fragments that are present in the blood of AMI patients and to quantify their relative abundance at different time periods after AMI.

## Materials and methods

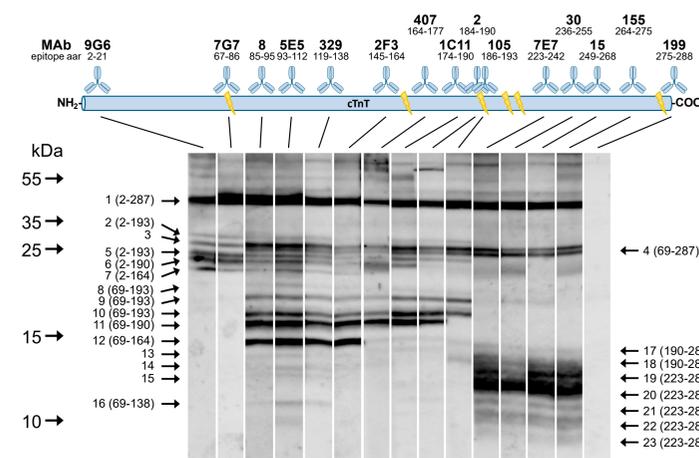
All of the patients selected for this study had an ST-elevation AMI that was followed by coronary angiography and stenting. Serial heparin plasma samples were collected from 23 AMI patients within 2 - 36 hours after the onset of chest pain.

cTnT and its fragments were immunoextracted from serum samples and then analyzed by Western blotting (WB) with monoclonal antibodies (MAbs) that were specific to the different epitopes of cTnT, as well as by three in-house sandwich immunoassays (329-406, 2A7-1A11 and 7G7-7E7) that utilized MAbs which were specific to different portions of the cTnT molecule.

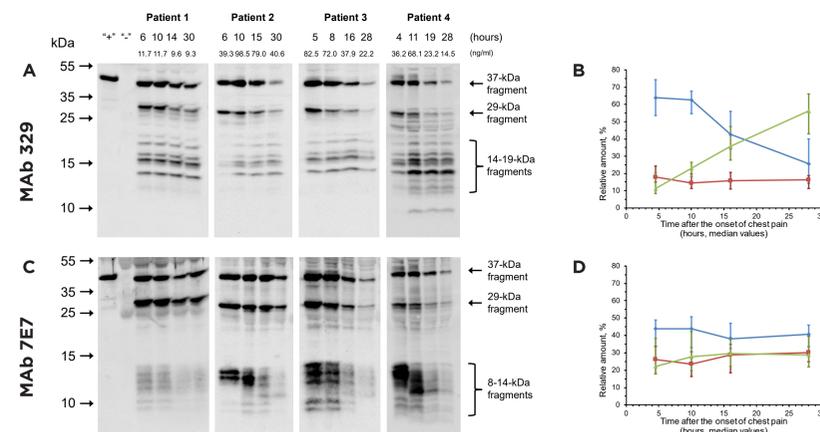
## Results and discussion

In the blood of AMI patients, we were able to discriminate 23 proteolytic fragments of cTnT with relative molecular masses of 10-30 kDa, along with "full-size" 2-287 amino acid residues (aar) cTnT of ~37 kDa (see Fig. 1). Four major regions of cTnT degradation were observed: the first one was between aar 68 and 69 and the second one was located near aar 164. The third region (which contained several sites of degradation) was approximately between aar 190 and 223. Lastly, all cTnT and its fragments that were detected in the blood had the last 288 Lys residue cleaved (immunostaining with the MAb 199 (see Fig. 1) and MS analysis, data not shown).

The immunostaining of cTnT from serial samples of AMI patients with the MAb 329 that is specific to the central part of the molecule (epitope 119-138 aar) revealed that the relative amount of the 37-kDa cTnT (2-287 aar) decreased by more than one-half from ~65% at the early time point (5±1.7 hours after AMI, mean±SD) to ~25% at the late time point (29±3.2 hours after AMI) (see Fig. 2 A and C). The relative amount of the 29-kDa fragment remained unchanged and represented ~15% of all detected cTnT. The relative amount of the 14-19 kDa central fragments increased from ~10% to ~55% (among those, the relative amount of the shortest fragment - which was approximately aar 69-164 - increased from ~5% to ~15%). Immunostaining of cTnT with the MAb 7E7 that is specific to the C-terminal part of the molecule (epitope 223-242 aar) revealed no considerable changes in the relative amount of the 37-kDa cTnT or shorter fragments over time (see Fig. 2 B and D).

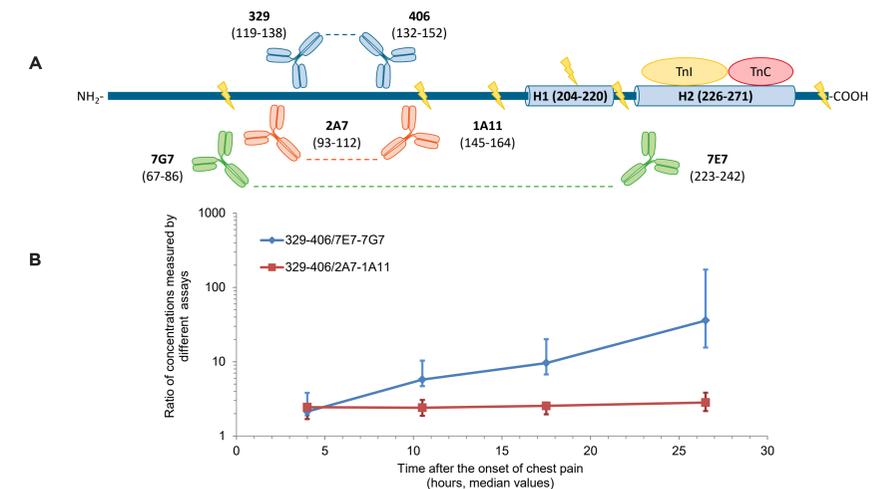


**FIGURE 1. WB studies of cTnT and its fragments that were immunoprecipitated from a heparin plasma sample of a representative AMI patient and stained by MAbs, which were specific to the different epitopes of cTnT.** The sample was taken 7 hours after the onset of chest pain, cTnT=26.2 ng/mL. The names of the MAbs used for staining and their epitopes are indicated above each strip. The approximate borders of each fragment are indicated in parenthesis on the sides of the figure.



**FIGURE 2. Changes in the relative amounts of cTnT fragments in heparin plasma samples of AMI patients over time.** A and C: Representative WB demonstrating the abundance of cTnT fragments in the serial heparin plasma samples of four AMI patients. The time after the onset of chest pain and the cTnT concentration in the initial samples are depicted above the lanes. Immunostaining was done with MAb 329 (A) or 7E7 (C). B and D: Median values of the relative amounts of different cTnT fragments from serial heparin plasma samples of 23 AMI patients that were taken in four time periods after AMI. Immunostaining was done with MAb 329 (B) or 7E7 (D). The whiskers represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles.

The ratio of concentrations measured in the serial samples of AMI patients (n=11) by the immunofluorescent assay (IFA) that utilized the MAbs 329-406 (capture-detection) to concentrations measured by the MAbs 2A7-1A11 increased 1.2 times (from 2.4 (1.7-2.6) to 2.8 (2.2-3.8), median (25<sup>th</sup> - 75<sup>th</sup> percentiles)) in the first 26 hours after AMI (see Fig. 3 B). During the same period of time, the ratio of concentrations measured by the MAbs 329-406 to concentrations measured by the MAbs 7E7-7G7 increased



**FIGURE 3. IFA studies of the dynamics of cTnT degradation.** A: A schematic representation of immunoassays used. B: Changes in the ratio of the concentrations of cTnT measured by the 329-406 to 7E7-7G7 (blue) and 329-406 to 2A7-1A11 (red) during the first 27 hours after AMI. The ratios were measured in serial heparin plasma samples of 11 AMI patients. The whiskers represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles.

~16.8 times (from 2.1 (1.9-3.8) to 35.9 (15.5-173.7)). This confirms the result of WB studies and indicates that (a) the region 69-164 is rather stable and (b) a substantial proteolysis of cTnT takes place either at site 68/69 or ~190-223, or at both sites.

Taken together, the data suggest that the region which is located approximately between aar 69 and 164 of cTnT is a promising target for the antibodies to be used in cTnT immunoassays. This is because in contrast to the C-terminal portion of cTnT it is not influenced by the binding of autoantibodies and it is not hindered by the other proteins of the ITC complex.

## Conclusions

- We have detected 23 different fragments of cTnT with relative molecular masses of 8-37 kDa in the blood of AMI patients.
- We have observed two major regions of proteolysis that are located approximately between aar 68 and 69, and between aar 190 and 223.
- During the first few hours (~5 hours after AMI) cTnT is present in the blood of patients mainly as a 37-kDa or as a 29-kDa fragment, whilst at later times (~30 hours after AMI) the amount of 12-16-kDa central fragments increases with the simultaneous decrease of the 37-kDa TnT.
- The central part of cTnT (approximately aar 69-164) appears to be the most stable part of the molecule and it is therefore a promising target for diagnostic antibodies. In contrast to the C-terminal portion of cTnT, it is not influenced by the binding of autoantibodies and it is not hindered by the other proteins of the ITC complex.