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Cardiac troponins in the blood of patients with acute myocardial infarction: in what forms do they exist?

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Introduction

The measurements of cardiac-specific Troponin I (cTnI) and Troponin T (cTnT) in the blood of patients are widely used for the diagnosis of acute myocardial infarction (AMI) because of the release of troponins from the damaged myocardium. The goal of this study was to find out in which forms cardiac troponins appear in the blood of patients after AMI onset.

Methods

HEPARIN PLASMA SAMPLES were taken from 5 AMI patients (all with STEMI) between 2 hours and 33 hours following the onset of chest pain. **GEL FILTRATION** studies (GF) were performed using an AKTA pure chromatography system on a HiLoad Superdex 200 PG 16/60 column (GE Healthcare). The resulting fractions were then analyzed for the presence of troponins by **SANDWICH IMMUNOFLUORESCENCE ASSAYS (FIA)** using Eu-labeled mAbs. All of the mAbs were from HyTest. The TnI19C7-TnI560 assay (capture/detection mAbs; epitopes 41-49/83-93 aar) detected cTnI in IC and ITC. The TnT329-TnT406 assay (119-138/132-152 aar) detected free cTnT and cTnT in ITC. The TnI84-TnI560 assay (117-126/83-93 aar) only detected cTnI in IC but did not detect cTnI in ITC. The "mixed sandwich assay" Tcom8-TnT7E7 permitted the detection of ITC only if all three of the troponins were bound together (the mAb Tcom8 recognized the structural epitope formed by cTnI and TnC polypeptide chains; the mAb TnT7E7 was specific to 223-242 aar of cTnT). **IMMUNOPRECIPITATION** of cTnI and/or cTnT and their fragments was performed on the sepharose CL-4B (GE Healthcare) conjugated with the mAbs that were specific to the different epitopes of the cTnI/cTnT molecule. **SEPARATION OF THE PROTEINS** was performed by means of 10-20% TRIS-GLY SDS PAGE with subsequent **WESTERN BLOTTING** (WB). The immunodetection of cTnI/cTnT and their fragments was performed by biotinylated mAbs that were specific to the different epitopes of the cTnI/cTnT molecules by means of ECL. The sensitivity of WB was not less than 10-15 pg per track.

Results

1. IC and ITC were found in the plasma of AMI patients. An additional ITC peak of lower molecular weight was also detected in the GF profile of AMI plasma samples and named low molecular weight ITC (LMW-ITC).

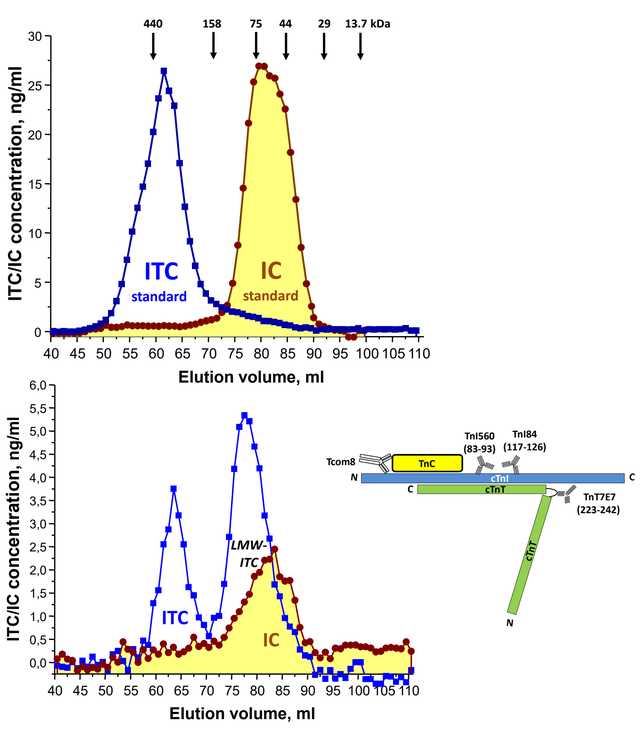


FIGURE 1. The GF immunoreactivity profiles of binary IC and ternary ITC complexes.
A. IC and ITC standards spiked into the heparin plasma samples of healthy individuals. The IC peak was detected by TnI84-TnI560 (-●-). The ITC peak was detected by Tcom8-TnT7E7 (-■-).
B. The GF profile of the AMI heparin plasma sample (9.5 hours following the onset of symptoms; cTnI concentration – 54 ng/ml, cTnT – 34 ng/ml).

2. ITC and LMW-ITC mainly contain full-size cTnI or slightly degraded cTnI from both ends. IC mostly contains cTnI truncated to approximately a 14 kDa fragment. ITC contains full-size cTnT or partially truncated cTnT. LMW-ITC contains primarily truncated cTnT with Mr of approximately 14 kDa.

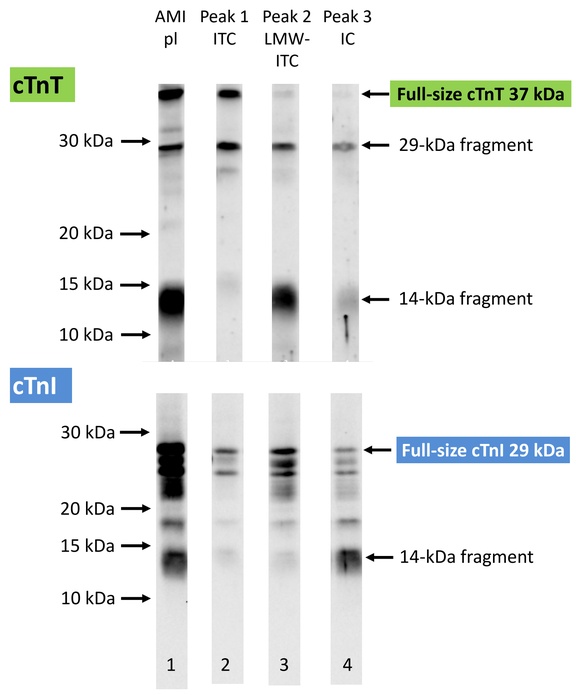


FIGURE 2. WB analysis of cTnI and cTnT in GF peaks of the AMI plasma sample.
Proteins were immunoextracted from the corresponding peaks on the mixture of anti-cTnI and anti-cTnT Sepharoses. cTnI was stained by TnI560, TnT – by TnT7E7
Track 1 – AMI plasma
Track 2 – GF peak 1 (ITC) contains 37-kDa cTnT (full-size) and its 29-kDa fragment; 29-kDa (full-size) cTnI and its 26-27 kDa fragments.
Track 3 – GF peak 2 (LMW-ITC) contains 29-kDa and mainly 14-kDa cTnT fragments; full-size cTnI and its 21-27 kDa fragments.
Track 4 – GF peak 3 (IC) contains cTnT fragment tails from the peak 2 and mainly 14-19 kDa fragments of cTnI.

3. LMW-ITC contains a cTnT C-terminal fragment of approximately 191-288 aar. A cleavage site is located between 191 and 223 aar of cTnT as the Tcom8-TnT7E7 assay (TnT epitope 223-242 aar detects both ITC and LMW-ITC, whereas the Tcom8-TnT1C11 assay (TnT epitope 171-190 aar) only detects ITC.

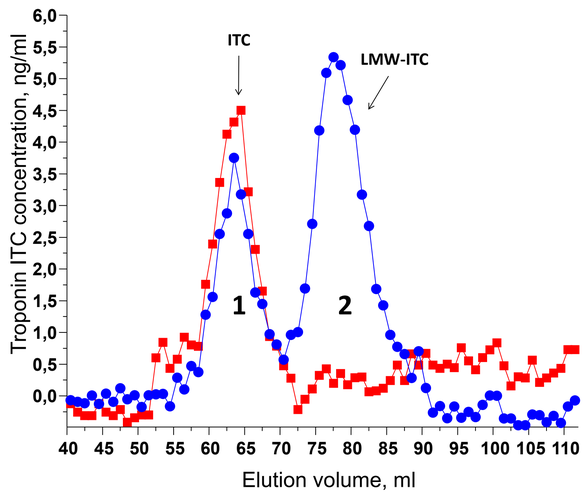
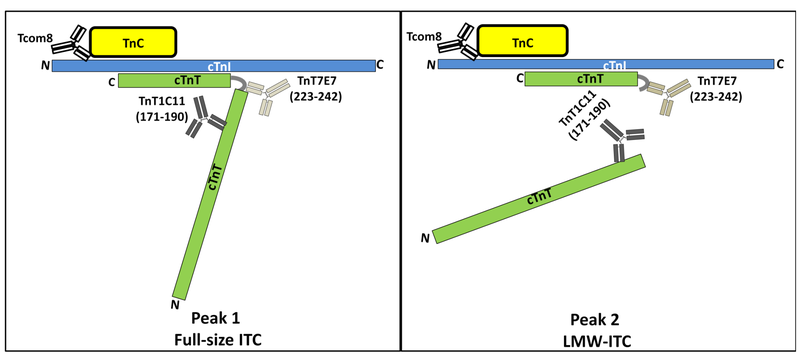
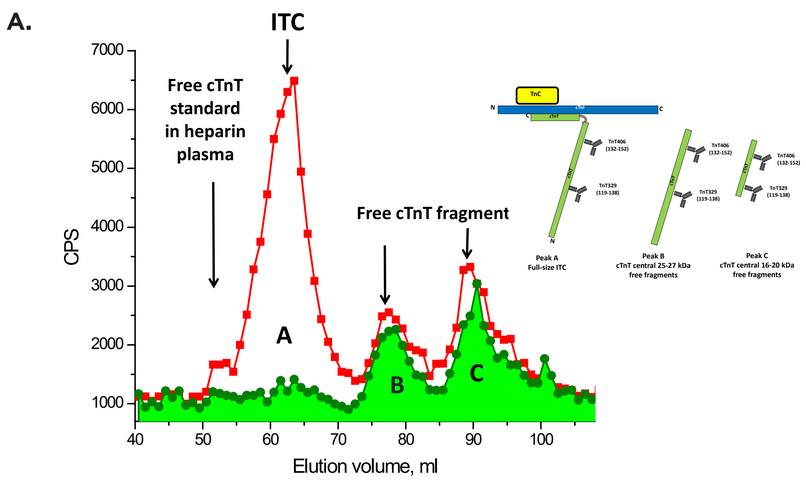


FIGURE 3. The GF immunoreactive peaks of ITC and LMW-ITC in AMI plasma samples detected by Tcom8-TnT7E7 and Tcom8-TnT1C11 assays.
Tcom8-TnT7E7 (-●-)
Tcom8-TnT1C11 (-■-)



4. AMI plasma samples were depleted of cTnT bound in complexes with cTnI using anti-cTnI Sepharose. The GF study shows that ITC and LMW-ITC peaks disappear in depleted samples, but two peaks of free cTnT fragments were left. Meanwhile, no free full-size cTnT were found. cTnT was immunoextracted on anti-cTnT Sepharose and stained either by TnT313 (119-138 aar) to the central region or TnT7E7 to the C-terminus.



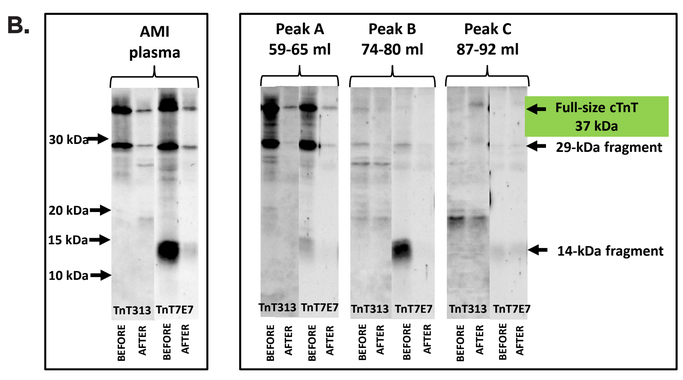


FIGURE 4. cTnT GF immunoreactivity profiles of AMI plasma samples before and after ITC and IC depletion.
A. The GF profiles of the AMI heparin plasma sample detected by the TnT329-TnT406 assay before (-■-) and after (-●-) ITC/IC depletion on anti-cTnI Sepharose. Peak A – ITC, Peaks B and C – free cTnT fragments.
B. WB analysis of the GF peaks before and after ITC/IC depletion.
"before" - before ITC/IC depletion on anti-cTnI Sepharose;
"after" - after ITC/IC depletion on anti-cTnI Sepharose.

Conclusions

We are able to demonstrate here that cardiac troponins can be found in three different complexes in the blood of AMI patients:
1) ITC that primarily contains full-size or slightly degraded cTnI, and full-size cTnT or its 29-kDa fragment;
2) LMW-ITC that contains a C-terminal region of cTnT starting after approximately 191-223 aars and slightly degraded cTnI.
3) IC that primarily contains cTnI, which is reduced to approximately 14-kDa fragments.

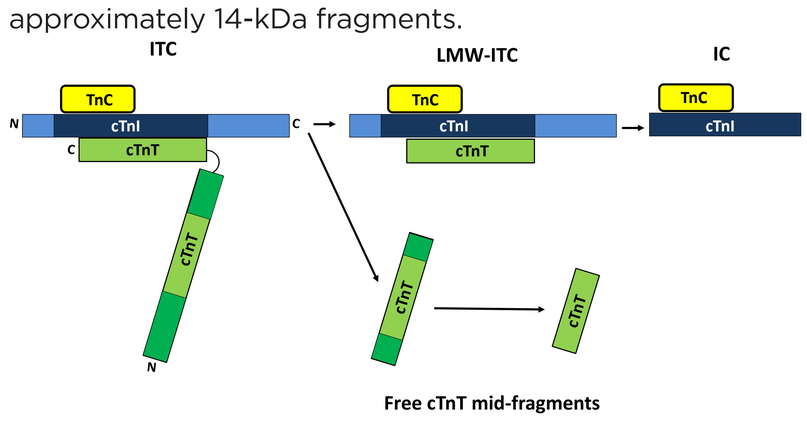


FIGURE 5. A presumptive scheme of the transformation of cardiac troponins from full-size molecules to low molecular forms.

Full-Size and Partially Truncated Cardiac Troponin Complexes in the Blood of Patients with Acute Myocardial Infarction
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