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Influence of heparin on the immunodetection of cardiac troponin

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Introduction

Ternary troponin complex (ITC) consists of three proteins: troponin I, troponin T and troponin C (TnC). ITC plays an important role in the regulation of muscle contraction. Cardiac isoforms of troponins T (cTnT) and I (cTnI) that are expressed exclusively in heart are actively used as biomarkers of various heart disorders, including acute myocardial infarction (AMI).

During the myocardial damage, different forms of cardiac troponins are released into the blood: full-size ternary complex (ITC), low-molecular weight ternary complex with only C-fragment of cTnT (LMW-ITC), binary cTnI-TnC complex (IC) (Fig. 1) and various proteolytic fragments of cTnT and cTnI [1, 2].

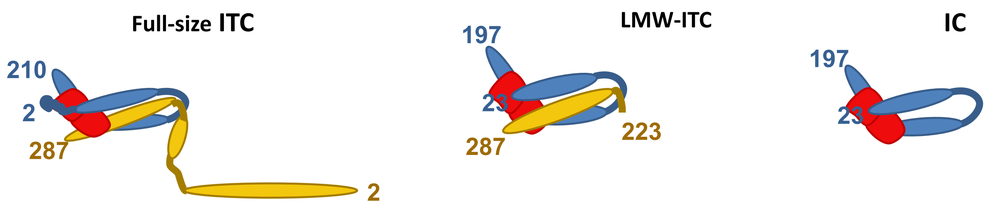

FIGURE 1. Schematic representation of cardiac troponin complexes that are present in AMI patients' blood. Red – TnC, yellow – cTnT, blue – cTnI. The numbers designate the terminal amino acid residues [2].

Heparin is a highly negatively charged polysaccharide that is used to prevent blood coagulation e.g. in the treatment of AMI and to prepare heparinized plasma samples that is utilized for immunodiagnostics. However, there is no consistent data describing how heparin interacts with different forms of troponins and how it affects the immunochemical measurement of these biomarkers in blood samples of AMI patients.

Here we studied the interaction of heparin with troponin complexes and its effect on the immunochemical determination of cardiac troponins.

Methods

Anti-TnT mAbs, human native and recombinant ITC, IC and TnT were from Hytest (Finland).

GEL FILTRATION PROCEDURE

Gel filtration studies (GF) were performed using an AKTA pure chromatography system (GE Healthcare). 1 μg of ITC, IC or free cTnT were dissolved in 1 mL of normal citrate or heparin plasma, loaded on a HiLoad Superdex 200 PG 16/60 column (GE Healthcare) and 1 mL fractions were collected and analyzed in immunoassays.

IMMUNOASSAYS

Cardiac troponins were measured using various sandwich fluoroimmunoassays (FIA) specific to different forms of cardiac troponins and troponin complexes. Conjugates of mAbs with stable Eu^{3+}-chelate were used for detection.

Results

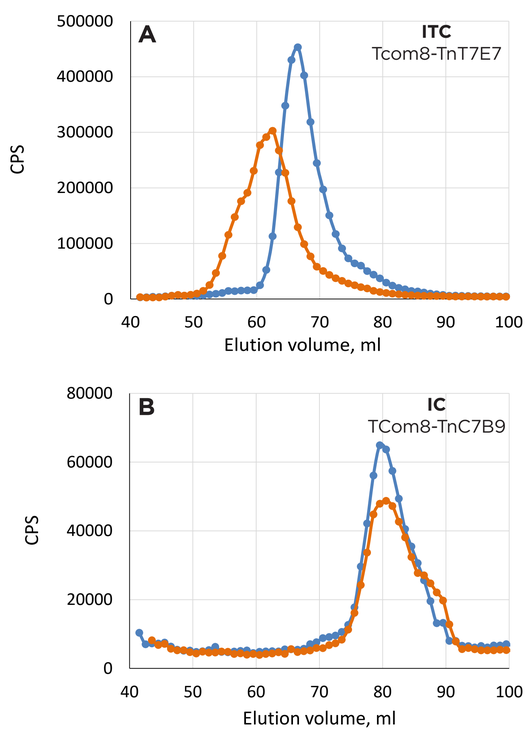

FIGURE 2. GF profiles of different forms of troponin complexes. (A – ITC, B – IC) and free cTnT (C) dissolved in normal heparin (orange) or citrate (blue) plasmas.

GF profile analyses showed that the peaks of ITC and free cTnT shifted to a higher molecular weight region in heparin plasma compared to citrate plasma (Fig. 2, A and C, respectively), whereas the peak of IC in heparin plasma eluted in the same volume as it was in citrate plasma (Fig. 2, B). This suggests that heparin binds to ITC via its cTnT component.

In the GF profiles of heparin and citrate plasma samples of AMI patients, the peak of the full-size ITC also shifted to a higher molecular weight region in heparin plasma whereas the peak of LMW-ITC eluted in the same volumes (Fig. 3). Since LMW-ITC contains only a C-terminal fragment of cTnT, we conclude that heparin binds to cTnT via its N-terminal and/or central part of the molecule.

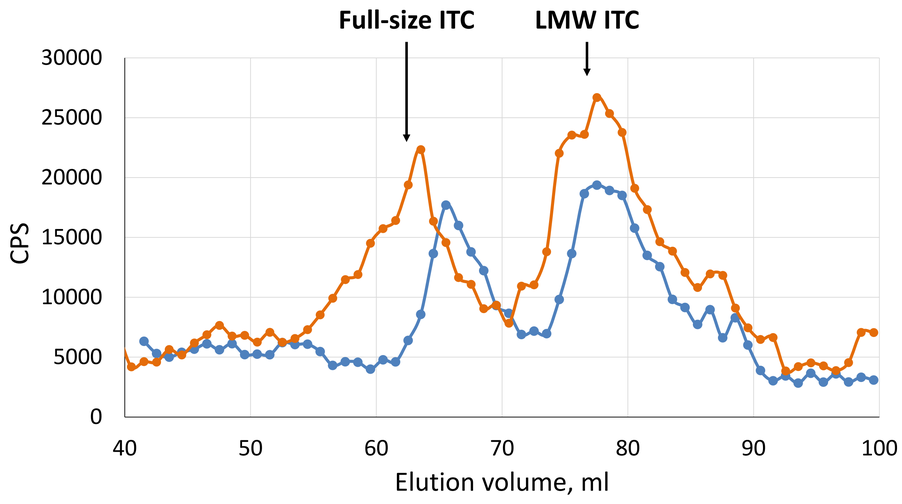

FIGURE 3. GF profiles of different forms of native troponin complexes in heparin (orange) and citrate (blue) plasmas of a representative AMI patient. Complexes were detected using Tcom8-TnT7E7 immunoassay.

To reveal the epitopes that affected by heparin on the cTnT molecule, we spiked ITC or free cTnT into citrate or heparin plasmas and compared the recognition of different cTnT epitopes by various anti-TnT mAbs in sandwich FIA. The recovery of spiked ITC and free cTnT by some antibodies was significantly lower in heparin plasma than in citrate plasma samples, which may indicate the sites of heparin binding. We identified four regions of cTnT that are affected by heparin (69-86 aar, 119-138 aar, 145-164 aar and 236-268 aar C-terminal part of free cTnT affected by heparin but undetectable in ITC) (Fig. 4).

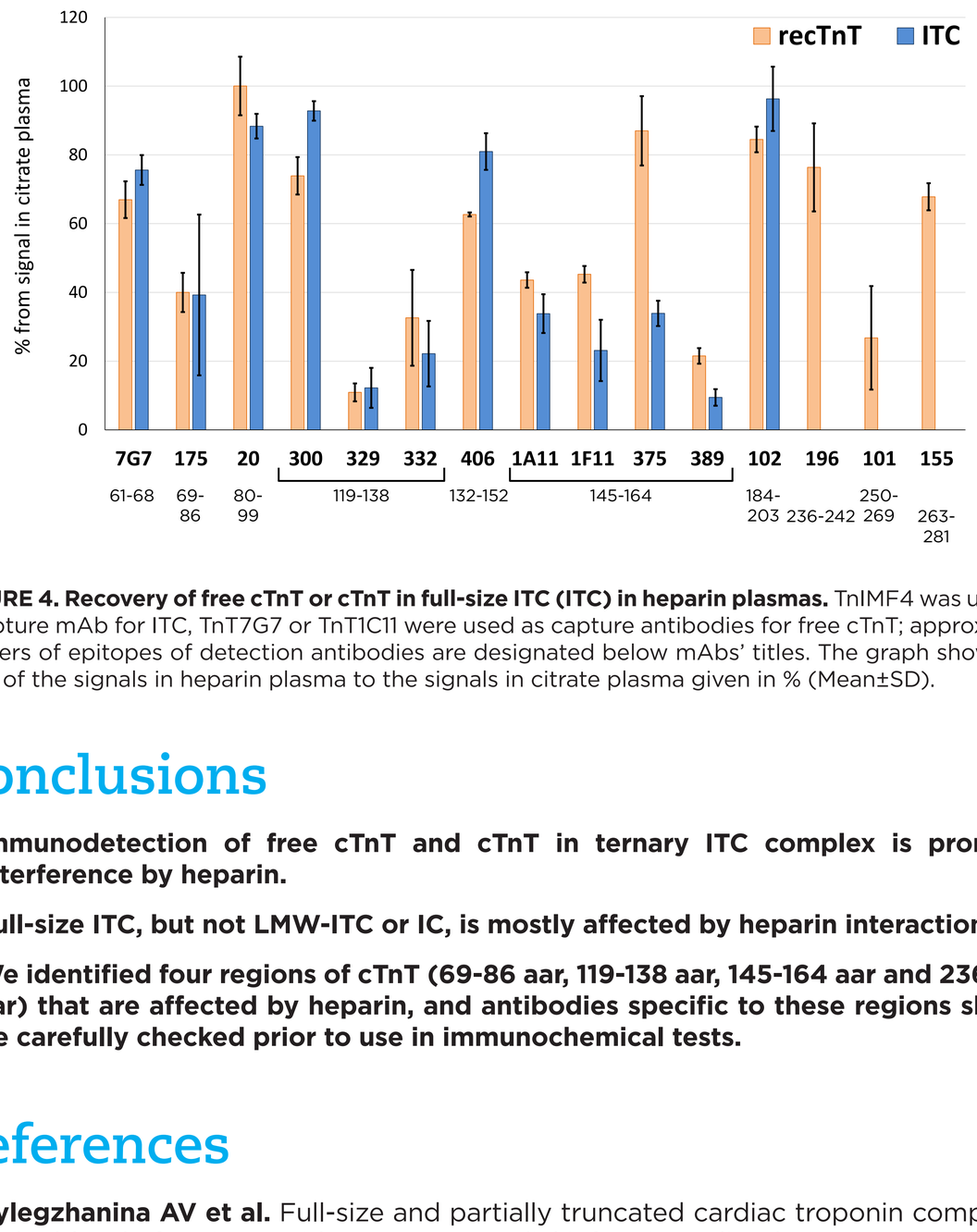

FIGURE 4. Recovery of free cTnT or cTnT in full-size ITC (ITC) in heparin plasmas. TnIMF4 was used as a capture mAb for ITC, TnT7G7 or TnT1C11 were used as capture antibodies for free cTnT; approximate borders of epitopes of detection antibodies are designated below mAbs' titles. The graph shows the ratio of the signals in heparin plasma to the signals in citrate plasma given in % (Mean±SD).

Conclusions

1. **Immunodetection of free cTnT and cTnT in ternary ITC complex is prone to interference by heparin.**
2. **Full-size ITC, but not LMW-ITC or IC, is mostly affected by heparin interaction.**
3. **We identified four regions of cTnT (69-86 aar, 119-138 aar, 145-164 aar and 236-268 aar) that are affected by heparin, and antibodies specific to these regions should be carefully checked prior to use in immunochemical tests.**

References

1. **Vylegzhanina AV et al.** Full-size and partially truncated cardiac troponin complexes in the blood of patients with acute myocardial infarction. Clin Chem. 2019;65:882–92.
2. **Katrukha IA, Katrukha AG.** Myocardial injury and the release of troponins I and T in the blood of patients. Clin Chem. 2021;67:124-130.