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The influence of troponin-specific autoantibodies on measurements of cardiac troponin I in binary and ternary complexes

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Introduction

Autoantibodies specific to cardiac troponins (TnAAbs) have been found in the blood of 6-7% of the population (1). It is believed that TnAAbs may negatively affect cardiac troponin I (cTnI) measurements in the blood of patients with acute myocardial infarction (AMI) by immunoassays that utilize monoclonal antibodies (mAbs) which are specific to certain epitopes located in the mid-part of the cTnI molecule (2). In this study, we investigated the epitope specificity of TnAAbs and their influence on measurements of cTnI presented in the sample in different forms.

Materials and methods

A total of 191 plasma samples were spiked with the ternary cTnI-cTnT-TnC complex and cTnI recovery was measured by using an immunoassay sensitive to the presence of TnAAbs (TnI19C7-TnI560). Twelve plasma samples that showed low cTnI recovery were selected and studied for TnAAbs epitope specificity. Meanwhile, cTnT recovery was measured in the same plasma samples. The mapping of sites on the cTnT that were affected by TnAAbs was performed by using eleven anti-cTnT mAbs specific to different epitopes. The gel filtration studies (GF) were performed in order to confirm the specificity of TnAAbs. The effect of TnAAbs on the measurements of the cTnI in the blood of AMI patients was analyzed after mixing TnAAbs-containing plasmas 1:1 with the plasma samples of AMI patients (n=35; cTnI concentrations from 2.5 to 35.1 µg/L).

Results and discussion

Subunit specificity of TnAAbs.
Three forms of cTnI (free cTnI, binary cTnI-TnC and ternary cTnI-cTnT-TnC complexes) were spiked into twelve TnAAbs-containing plasma samples at the concentration of 50 Qg/L and cTnI recoveries were analyzed. The well-pronounced inhibitory effect of TnAAbs on cTnI measurements was only observed for the ternary complex (mean recovery 10.3%) (see Fig.1). The inhibitory effect was significantly less pronounced for cTnI-TnC binary complex or free cTnI (mean recoveries: 71.0% and 96.5% respectively).

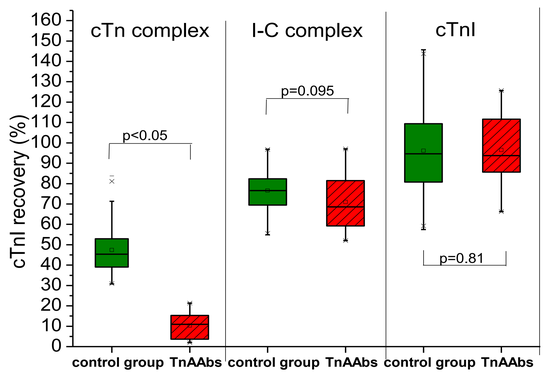

FIGURE 1. Recoveries of three cTnI forms spiked into the TnAAbs-containing and control plasmas. The ternary cTnI-cTnT-TnC complex, binary cTnI-TnC complex and free cTnI were spiked into the control (n=179) or TnAAbs-containing plasma samples (n=12) at a concentration of 50 Qg/L and were then measured by the TnI19C7-TnI560 immunoassay.

cTnT epitope sensitive to TnAAbs.
The mapping of sites on the cTnT that are affected by TnAAbs showed that only one epitope (223-242 aar) of cTnT was significantly influenced by TnAAbs (see Fig. 2).
The inhibitory effect of TnAAbs on cTnT detection was only found when ternary cTnI-cTnT-TnC complex was spiked into plasma (mean recovery 14%), whereas it was much less pronounced when free cTnT was spiked (mean recovery 73%).

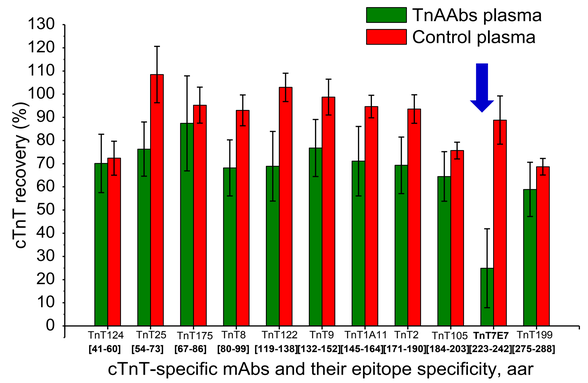

FIGURE 2. Recovery of cTnT (in the form of the ternary cTnI-cTnT-TnC complex) spiked into the TnAAbs-containing and control plasma samples. The ternary cTnI-cTnT-TnC complex was spiked into the control (n=12) and TnAAbs-containing plasmas (n=12), and then measured by immunoassays that utilized the mAb TnI625 as the capture antibody and various anti-cTnT mAbs, specific to different epitopes (designated in brackets), as detection antibodies.

As the inhibitory effects of TnAAbs on the detection of both cTnI and cTnT were only observed for the cTnI-cTnT-TnC complex, we suggest that TnAAbs are specific to structural epitopes that are formed by closely located cTnI and cTnT polypeptide chains (see Fig. 3).

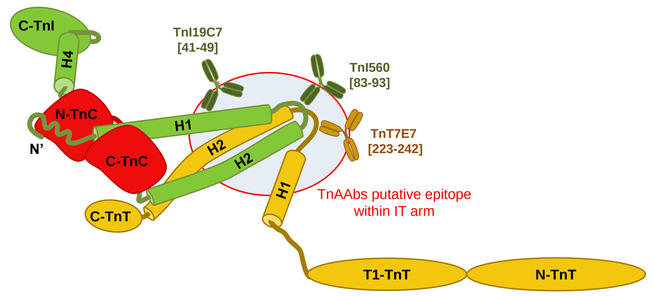

FIGURE 3. Structure of the ternary cTnI-cTnT-TnC complex. Adapted from [3].

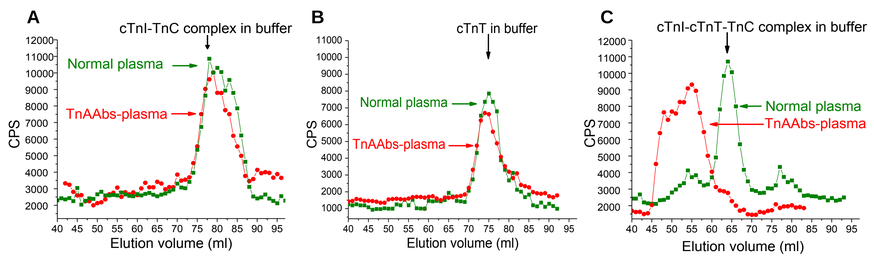

FIGURE 4. Representative GF profiles of the binary cTnI-TnC complex (A), free cTnT (B) and ternary cTnI-cTnT-TnC complex (C) spiked into the TnAAbs-containing (-●-) and control plasma samples (-■-). The immunoreactivity of the cTnI in the ternary cTnI-cTnT-TnC and binary cTnI-TnC complexes was measured by the TnI625-TnIMF4 immunoassay in twelve TnAAbs-containing and six control plasma samples. The immunoreactivity of free cTnT was measured by the TnT9-TnT1A11 immunoassay. Neither assay was sensitive to the presence of TnAAbs in the sample.

GF studies of TnAAbs specificity
Samples were analyzed by the GF method in order to confirm that TnAAbs only interact with the ternary cTnI-cTnT-TnC complex and do not recognize the cTnI-TnC binary complex or free cTnT.
Free cTnT, cTnI-cTnT-TnC complex or cTnI-TnC binary complex was spiked into the TnAAbs-containing or into the control plasma samples. The immunological activity of the cTnI and cTnT in the fractions was detected after GF with the TnAAbs-insensitive assays. The retention volumes for the cTnI-cTnT-TnC peak in twelve TnAAbs-containing plasma samples shifted to the area of higher molecular weight proteins as compared to the control samples (see Fig. 4). It was concluded that TnAAbs only form the high molecular weight complex with the cTnI-cTnT-TnC complex and not with the binary cTnI-TnC complex or free cTnT.

TnAAbs and the measurements of endogenous troponins
Serial plasma samples of AMI patients were collected 4-9, 10-19 and 22-36 hours following the onset of symptoms (cTnI concentrations varied from 2.5 to 35.1 µg/L) and were mixed with four different TnAAbs plasma samples. It was found that the negative influence of TnAAbs on the cTnI recovery measured by the TnI19C7-TnI560 assay was most pronounced during the first hours after the onset of symptoms and became weaker at later stages. Meanwhile, the negative effect of TnAAbs on the measurements of endogenous cTnI in AMI samples was less pronounced as compared to measurements of cTnI spiked in the form of ternary cTnI-cTnT-TnC complex (see Fig. 5).

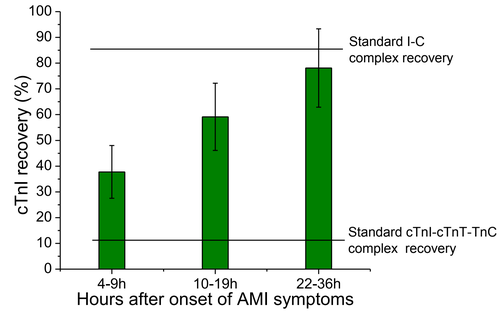

FIGURE 5. Influence of TnAAbs on cTnI recovery in the plasma samples of AMI patients at different times following the onset of AMI symptoms. Each of the samples of the AMI patients (six patients; three time points for each patient) was mixed 1:1 with four individual TnAAbs-containing plasma samples. Recoveries were measured by utilizing the TnI19C7-TnI560 immunoassay.

Conclusions

1. TnAAbs are not specific to the cTnI per se but instead to the structural epitopes formed by cTnI and cTnT polypeptide chains

2. The effect of TnAAbs on cTnI measurements is less pronounced in AMI blood samples than in the samples with spiked ternary cTnI-cTnT-TnC complex

3. The negative effect of TnAAbs on cTnI measurements is more noticeable in the early AMI samples (first few hours after the event) rather than in the late AMI samples

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