INTRODUCTION
Cardiac isoform of troponin I (cTnI) is a recognized marker of myocardial cell damage. As it was shown before in patient’s blood the major part (if not all) of cTnI exists as a binary complex with troponin C (TnC). Recently it was shown that cTnI concentration in some samples could be seriously underestimated because of the presence of cTnI-specific autoantibodies. Negative effect of autoantibodies on cTnI measurements is especially important in the case of minor cardiac events, when cTnI concentration in the sample is low. Measurements of such samples by conventional cTnI immunoassays can result in false negative diagnosis. Here we report completely new type of cTnI immunoassay with minor susceptibility to the presence of cTnI specific autoantibodies.

METHODS
Monoclonal antibodies

Monoclonal antibodies were generated after mice immunization by human cardiac Tn complex. New MAb TCom8 is specific to the native cardiac Tn complex and does not recognize neither free cTnI, nor TnC, and cTnT. MAb 7B9 is TnC-specific.

Immuonassay

Two-step "sandwich"-immunoassay was performed in 96-well plates. At the 1-st step unlabelled capture MAb was incubated for 30 min at room temperature with gentle shaking. After washing the mixture of detection MAb (labeled with stable Eu³⁺ chelate) and serum sample or calibrator was added for 30 minutes. After washing 0.2 ml of LANFIA enhancement solution per well was added. The plate was shaken for 3 min and fluorescence was measured using Victor Plate Fluorimeter (Wallac, Turku, Finland). Sensitivity of the new assay was better than 10 pg/ml in case human cardiac troponin I complex (SRM 2921 international standard) is used as a calibrator. (Principle of new cTnI assay is presented on Figure 1)

RESULTS

New cTnI assay was compared with two generations of cTnI conventional assays. First generation assay, utilizing two MAbs, specific to the central part of cTnI molecule (MAbs 19C7 and 560) and second generation assay – utilizing two capture (M18 and 560) and two detection (19C7 and MF4) cTnI-specific MAbs.

It was shown, that new type of TnI assay displays significantly higher sensitivity to the antigen in comparison with two others, used in the study (Figure 2)

Different additives and antigen modifications did not influence significantly the antigen recognition by three tested assays, with the only one exception – EDTA addition in the case of new assay. (Figure 3) EDTA is known to change (decrease) the affinity of interaction between TnC and TnI subunits in troponin complex by changing conformation of TnC molecule. This, possibly, results in decreased affinity of interaction of MAb TCom8 with the antigen. That fact makes EDTA undesirable for the preparation of the samples to be measured by new cTnI assay.

When antigen was spiked in the blood samples of donors, containing high and medium level of anti-cTnI autoantibodies, we observed significantly better recovery in case of new assay than in case of tested in the study conventional cTnI assays, utilizing all MAbs specific to cTnI molecule (Figure 4)

Preliminary clinical trials revealed that in the blood samples from the patients with minor cardiac events new assay is able to detect more cTnI than the best tested conventional cTnI assays (Figure 5).

CONCLUSIONS.

Here we report about development of new type of sensitive and specific cTnI assay displaying reduced susceptibility to the presence of cTnI-specific autoantibodies.

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