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Skeletal troponin I detection in the blood of patients with skeletal muscle injury

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Introduction

Troponin I together with troponin T and troponin C (TnC) forms the troponin complex, which regulates muscle contraction. Human TnI is represented by three isoforms – cardiac (cTnI) and fast and slow skeletal (fsTnI and ssTnI respectively, Fig. 1). FsTnI and ssTnI could be used as highly specific markers of skeletal muscle injury because they are specific to skeletal muscle but not to myocardium.

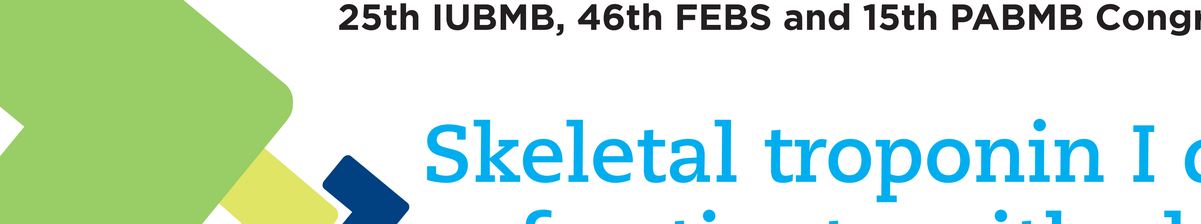


FIGURE 1. Alignment of three isoforms of human TnI. Identical amino acid residues are marked in blue. Epitopes of the antibodies are marked with rectangles.

cTnI exists in the blood of patients not as a free protein but in association with other troponins: TnC (binary IC complex) and with TnT and TnC (ternary ITC complex) [1]. This knowledge is crucial for the development of accurate diagnostic immunoassays because some epitopes of cTnI are shielded in those complexes. There are no data whether skTnIs exist in blood of patients as free proteins or in complexes and if so, which epitopes are shielded.

The aim of the study was to develop immunoassays to detect fsTnI and ssTnI separately or together and to investigate in what form skTnIs exist in blood of patients.

Material and methods

- Monoclonal antibodies (mAbs), recombinant fsTnI, ssTnI and troponin complexes: IC and ITC (HyTest).
- In two-step sandwich fluoroimmunoassays (FIA) Eu-labeled specific mAbs were used for detection.
- Western Blotting (WB) was performed utilizing biotinylated mAbs and streptavidin-polyHRP (Pierce) with further ECL detection (ECL Clarity kit, BioRad).
- Gel filtration (GF) studies were performed on AKTA pure chromatography system (GE Healthcare) with HiLoad Superdex 200 PG 16/60 column (GE Healthcare).
- Serum samples of patients who underwent bone reconstruction surgery were taken 20-30 hours after the intervention.

Results

Selection of anti-skTnI in WB

From a large panel of mAbs raised against skTnIs we selected three antibodies with specificities to the various skTnI isoforms:
- mAb skTnI58, specific to ssTnI;
- mAb skTnI89, specific to fsTnI;
- mAb skTnI50 stained both skeletal isoforms of TnI, but not cTnI (Fig. 2).

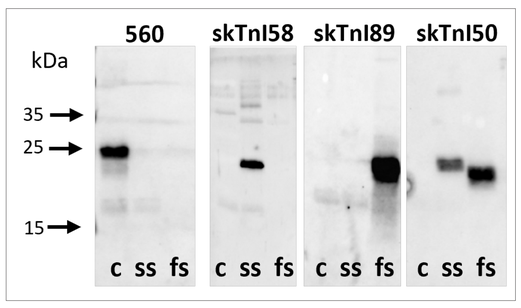
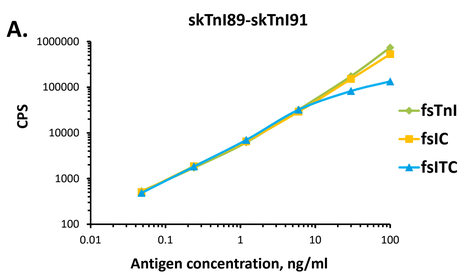
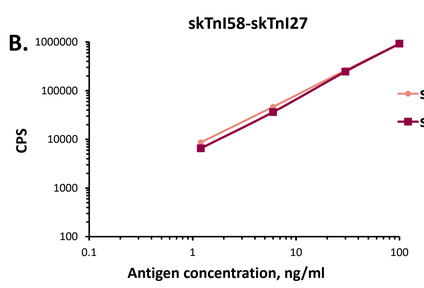


FIGURE 2. Determination of specificity of anti-skTnI mAbs by means of WB.
mAbs: 560 (specific to cTnI); skTnI58; skTnI89; skTnI50
Antigens: cITC (c), ssITC (ss), fsITC (fs)

FIAs that specifically detect skeletal isoforms of TnI

Assays that can selectively detect both free and complexed TnI were developed:
Anti-fsTnI: skTnI89-skTnI91 cross-reactivity with other TnI isoforms was <0,1%.
Anti-ssTnI: skTnI58-skTnI27, cross-reactivity with other TnI isoforms was <0,05%.
Anti-fsTnI and ssTnI: skTnI29-skTnI50, cross-reactivity with cTnI was <0,5%.





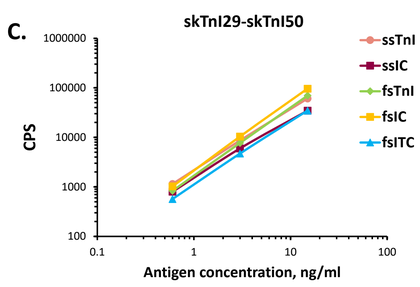
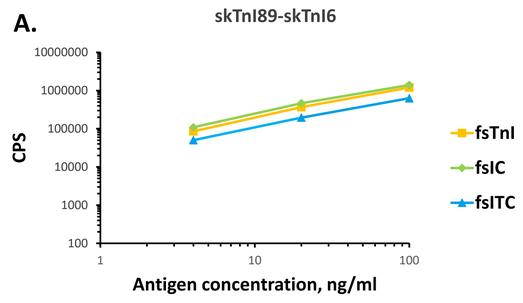
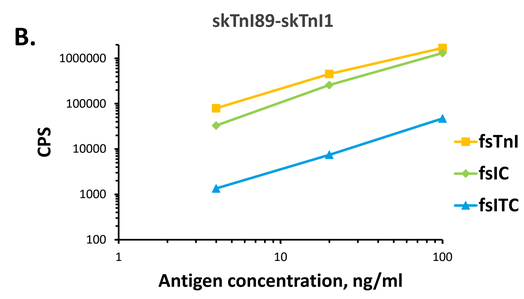


FIGURE 3. Sandwich-FIAs for skTnI detection:
A – skTnI89-skTnI91 (fsTnI-specific)
B – skTnI58-skTnI27 (ssTnI-specific)
C – skTnI29-skTnI50 (specific for both fs and ssTnI)

FIAs that detect different forms of fsTnI

Next, we developed prototype FIAs that recognize fsTnI in different complexes (Fig. 4).





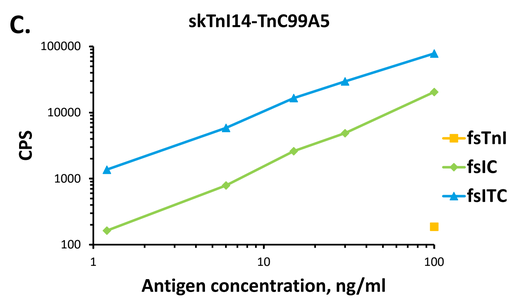
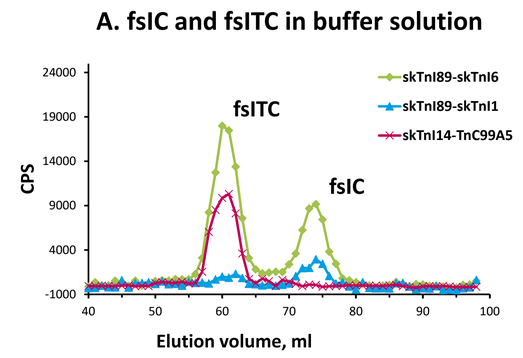
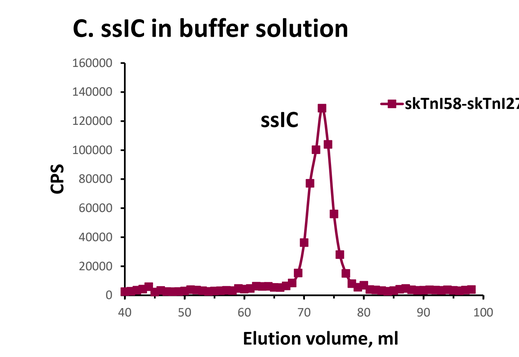


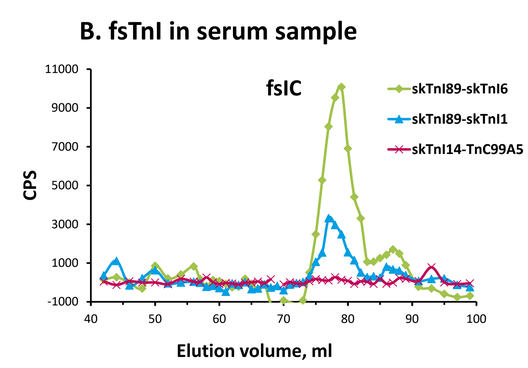
FIGURE 4. Sandwich-FIAs, recognizing different forms of fsTnI.
A – skTnI89-skTnI6 detects fsTnI, fsIC and fsITC
B – skTnI89-skTnI1 detects fsTnI and fsIC
C – skTnI14-TnC99A5 (TnC99A5 is specific to TnC) detects fsITC only

Investigation of fsTnI/ssTnI forms in blood of patients with skeletal muscle injury

In the serum samples that were taken 20-30 hours after surgical interventions, both fsTnI and ssTnI were present as binary IC complexes, but not as ternary ITC complexes or free TnI, (see GF profiles of a representative sample in Fig, 5, B and D).







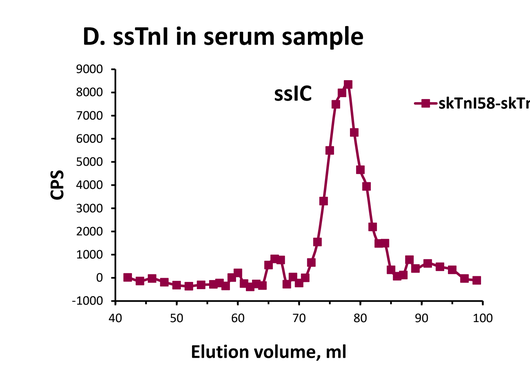


FIGURE 5. Detection of skTnI in blood samples of patients with skeletal muscle injury.
A and B – fsTnI detection, C and D – ssTnI detection.
A – artificial fsITC and fsIC in buffer solution; B – serum of patient taken 28 hours after surgery.
C – artificial ssIC in buffer solution; D – serum of patient taken 28 hours after surgery

skTnIs release in blood during skeletal muscle injuries of different etiologies (traumas, hereditary and congenital muscle diseases, etc.). The use of immunoassays, which are capable to recognize both skeletal isoforms of TnI, enables the most complete detection of skTnI present in the blood of patients. Immunoassays that selectively detect fsTnI and ssTnI makes it possible to the differential diagnostic of skeletal muscle diseases which differ in the type of damaged fibers (fast- or slow-type).

Conclusions

- **We developed fluoroimmunoassays that detect both isoforms of skTnI with no cross-reactivity with cTnI and assays that detect only ssTnI or fsTnI.**
- **We developed fluoroimmunoassays recognizing complexed and free fsTnI.**
- **20-30 hours after skeletal muscle injury, fsTnI and ssTnI are present in the blood of patients as binary complexes with TnC.**

References

1. **Katrukha IA and Katrukha AG,** 2021, ClinChem 67(1), p. 124-130