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SKELETAL TROPONIN I ISOFORMS: IN WHAT FORMS DO THEY EXIST IN HUMAN BLOOD?

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INTRODUCTION

Troponin I (TnI) is a thin filament protein which, together with troponin T (TnT) and troponin C (TnC), forms the troponin complex and regulates muscle contraction (**Fig. 1**). In humans, there are three isoforms of TnI: cardiac TnI (cTnI), which is expressed only in the heart, and two skeletal – fast (fsTnI) and slow (ssTnI) skeletal – which are present in fast-twitch and slow-twitch muscles, respectively.

While cTnI is widely used for the determination of myocardial damage [1], skeletal TnIs could be utilized as promising biomarkers of skeletal muscle diseases. In contrast to currently used proteins (creatine kinase and myoglobin), they are strictly specific to skeletal muscles [2, 3]. To develop reliable immunodetection methods, it is important to determine the forms in which skeletal TnIs exist in the blood of patients: are they present as free proteins or are they complexed with TnC (IC) or with TnC and TnT (ITC). It is also necessary to find out whether skeletal TnIs appear in blood as full-sized proteins or as partially proteolyzed fragments.

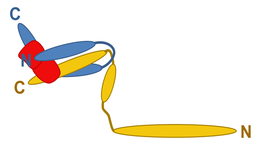

Figure 1. Schematic representation of TnI-TnT-TnC (ITC) ternary complex [1]. Blue – TnI, Yellow – TnT, Red – TnC.

Aim of the study: to investigate skeletal TnIs present in the blood of patients with skeletal muscle injuries.

MATERIALS AND METHODS

All monoclonal antibodies (mAbs) and troponins were provided by Hytest.

Sera samples were collected 24 hours after hip endoprosthesis surgery and subjected to gel filtration (GF). GF was performed on the AKTA pure chromatography system (GE Healthcare) with the HiLoad Superdex 200 pg 16/60 column (GE Healthcare). The fractions obtained were subsequently analyzed by various sandwich fluoroimmunoassays that recognize only fsITC (skTnI14-TnC99A5), only ssITC (TnT111-skTnI38), both free and complexed fsTnI (skTnI89-skTnI6), or free and complexed ssTnI (skTnI58-skTnI27).

Skeletal troponins were extracted from the sera samples using affinity matrices utilizing different mAbs that were specific to the various epitopes of fsTnI or ssTnI. Probes were further subjected to Western blotting (WB), and troponins were visualized using anti-fsTnI mAbs recognizing the central (skTnI89, epitope 86-105) or C-terminal (skTnI11, 142-161) portions of the protein; mAbs that were specific to the central (skTnI77, 44-63) or C-terminal (skTnI38, 170-187) parts of ssTnI; TnT-specific mAb (TnT111).

CONCLUSIONS

- **24 hours after skeletal muscle injury, both fsTnI and ssTnI are present in human blood as binary complexes with TnC.**
- **Both fsTnI and ssTnI undergo partial proteolysis; while ssTnI is cleaved at the C-terminal part of the molecule, fsTnI is likely to be partially cleaved at its N-terminus.**

RESULTS

On GF profiles of sera samples taken 24 hours after hip endoprosthesis surgery, major peaks with an elution volume of ~78 ml corresponding to binary fsIC and ssIC were recognized while no fsITC nor ssITC were observed (**Fig. 2**).

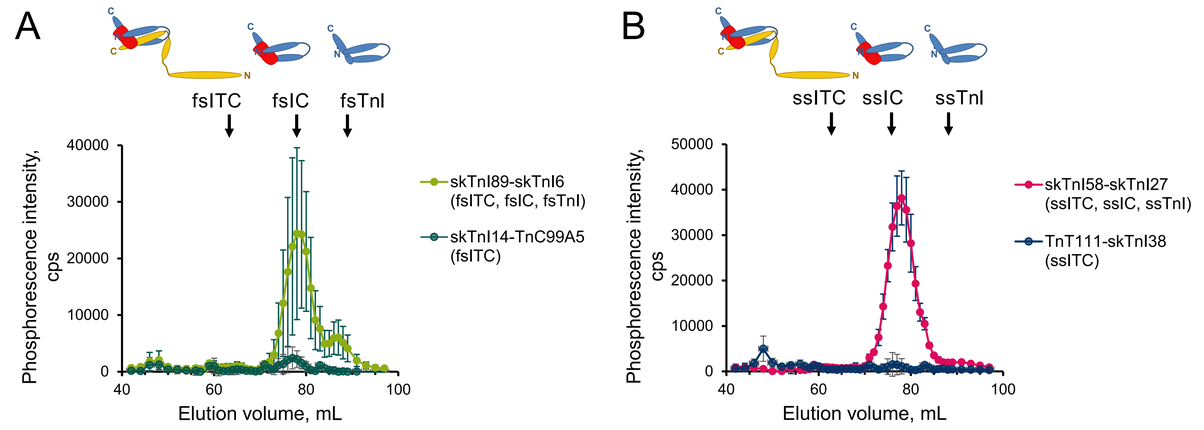

Figure 2. GF profiles of the sera samples of patients analyzed by FIAs specific to fast (A) and slow (B) skeletal troponin isoforms, mean ± SD.

By means of WB of the same sera samples, fsTnI was shown to be present as a major full-size band and proteolyzed fragments of 12-20 kDa, the latter being detected by fsTnI-specific mAbs recognizing both central (skTnI89) and C-terminal (skTnI11) parts of the molecule (**Fig. 3A**). ssTnI was also present by a major full-size band and proteolyzed fragments of 12-20 kDa. However, in contrast to fsTnI, fragments were visualized only by the mAb skTnI77 that was specific to the central part of the molecule (**Fig. 3B**).

Neither fsTnT nor ssTnT was detected in these probes, which confirms the absence of ITC complex in the studied sera samples (**Fig. 3**).

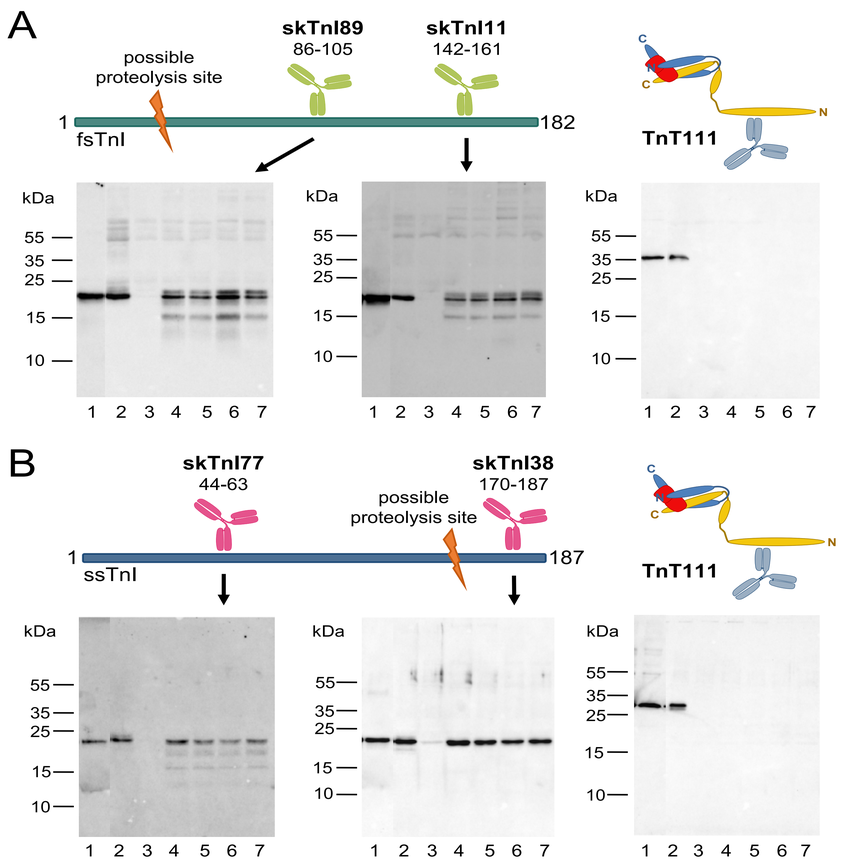

Figure 3. fsTnI (A) and ssTnI (B) extracted from the sera of the patients and visualized with mAbs.

1 – Positive control: fsITC (A) or ssITC (B);
2 – Human skeletal muscle extract in normal human serum (NHS);
3 – Negative control: NHS;
4-7 – Troponins extracted from the sera samples of four representative patients.

REFERENCES

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3. Bogomolova AP, Katrukha IA, Emelin AM, Zabolotsky AI, Bereznikova AV, Lebedeva OS, Deev RV, and Katrukha AG. Development of immunochemical systems for detection of human skeletal troponin I isoforms. *Biochemistry (Moscow) 2025 Mar; 90(3): 349-363*