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Human cardiac TnI degradation and antibody selection for the assay development

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Introduction

The immunological measurement of cardiac troponin I (cTnI) in blood is one of the most trusted methods of acute myocardial infarction (AMI) diagnosis. However, in spite of a long history of this biomarker in clinical practice, the selection of proper antibodies for cTnI assays remains a complex task. The first in vitro studies showed that cTnI is prone to degradation from both the N- and C-terminus with the ~30-130 aar being the most stable part of the molecule [1]. Meanwhile, later studies revealed that the blood of some patients contained autoantibodies which negatively interfere with most of the immunoassay mAbs that are specific to the central (~40-130 amino acid residues, aar) fragment of cTnI [2]. In the current study we aimed: a) to analyze the dynamics of cTnI degradation following AMI; and b) to border cTnI fragments that are present in the circulation of AMI patients in order to determine the epitopes of antibodies which are not significantly influenced both by the proteolytic degradation and autoantibody interference.

Materials and methods

All of the patients who were selected for this study had an ST-elevation myocardial infarction which was followed by coronary angiography and stenting. Serial serum samples were collected within a period of 2 - 36 hours following the onset of chest pain.

cTnI and its fragments were immunoextracted from serum samples and analyzed by Western blotting (WB) with monoclonal antibodies (mAbs) that were specific to the different epitopes of cTnI and by two in-house sandwich immunoassays (MF4-4C2 and 19C7-560) which were specific to the terminal and central parts of cTnI respectively.

In order to quantify the amount of cTnI that could be detected in WB by mAbs that were specific to the different parts of the molecule, the results of the fragment mapping by 15 mAbs were combined with the results of quantification of relative abundance of different cTnI fragments following staining by the mAb 560, which is capable of interacting with all detectable fragments with the same level of intensity. Following this, the fraction of cTnI that each mAb was capable of interacting with was calculated.

Results and discussion

We were able to detect 12 bands that corresponded to the full-sized cTnI and its 11 fragments. The relative abundance of cTnI and its fragments did not change considerably over time (see Figs 1A and B) following AMI. Studies of serum samples by means of FIA showed only a twofold fall in terms of the ratio of measured concentrations within the 36 hour period following the onset of AMI (see Fig. 2), confirming that no dramatic degradation of cTnI takes place in the first 36 hours following AMI.

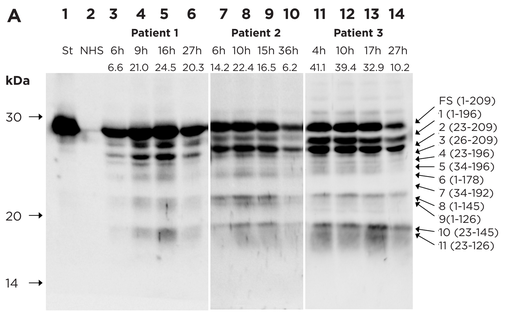

FIGURE 1. Change of cTnI fragment ratios in serum samples of AMI patients over time.
A: Full-size (FS) cTnI and its fragments (1-11) in the serial serum samples of three AMI patients taken after stenting. The time after the onset of chest pain and cTnI concentration in the initial samples (ng/ml) is indicated below the lane number. Staining with the HRP-labeled mAb 560. Lane 1: cTnI standard (recTnI). Lane 2: Negative control (immunoextraction of proteins from NHS). Lanes 3-14: cTnI immunoextracted from serial serum samples of three representative AMI patients.
B: Changes of the ratio of different cTnI fragments from serial serum samples (median values for 26 AMI patients). Whiskers are shown for the ratio of the three most abundant bands (Full-size molecule, Fragment 2 and Fragments 10 and 11) and these represent the 25th and 75th percentiles.

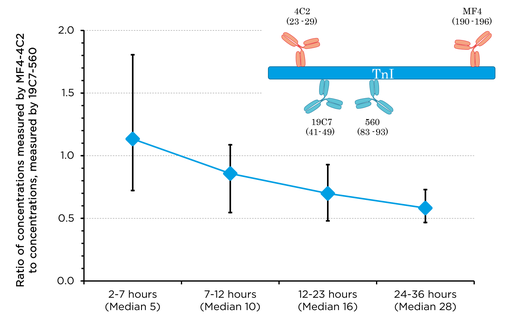

FIGURE 2. cTnI degradation as measured by FIA.
Changes in the median values of ratio of cTnI concentrations in the samples of 64 AMI patients measured with the assay specific to the terminal portions of cTnI (**MF4-4C2**) to the concentration, measured by the assay specific to the stable central portion of the cTnI molecule (**19C7-560**).

Quantification of the amount of cTnI that could be detected by the different mAbs in the blood of AMI patients showed that the very ends of cTnI (both the N- and C-terminal) were prone to degradation. However, starting with the mAb 4C2 (aar 23-29) antibody, which is specific to the N-terminal region of cTnI, ≥90% of all detectable cTnI and its fragments was recognized (see Table 1). From the C-terminus, the mAb MF4 (aar 190-196) interacted with ~80% of all extracted cTnI. mAbs that were specific to the epitopes located approximately between amino acid residues (aar) 34 and 126 stained all extracted cTnI. Therefore, mAbs with epitopes that fall within aar 23-196 recognized ≥ 80% of all cTnI detected in the samples of patients that were taken within the first 36 hours following AMI.

TABLE 1. Relative amount of cTnI and its fragments that could be detected by different mAbs in serial serum samples of AMI patients (n=26). Mean %±SD.

mAb	Epitope (aar)	Fragments that comprise the epitope	Amount of cTnI (%) stained by mAb 560			
			6 hours	10 hours	15 hours	28 hours
9F6	1-15	FS, 1, 6, 8, 9	58.9±12.3	56.9±10.8	54.2±10.1	53.4±12.8
909	18-22	FS, 1, 6, 8, 9	58.9±12.3	56.9±10.8	54.2±10.1	53.4±12.8
4C2	23-29	FS, 1, 2, 4, 6, 8-11	94.0±2.2	93.8±2.5	93.9±2.6	93.5±3.1
M155	26-35	FS, 1-4, 6, 8-11	95.5±2.2	95.5±2.0	95.5±1.6	95.7±1.6
10F4, 19C7, 560, 16A11, 84	34-126	All detected fragments	100	100	100	100
M46	130-145	FS, 1-10	95.4±2.5	95.3±1.8	94.2±2.4	93.8±2.5
441	148-158	FS, 1-7	86.0±6.6	85.9±5.0	83.8±5.7	83.0±6.6
625	169-178	FS, 1-7	86.0±6.6	85.9±5.0	83.8±5.7	83.0±6.6
C5	186-192	FS, 1-5, 7	85.5±6.5	85.3±5.1	83.1±5.7	82.5±6.3
MF4	190-196	FS, 1-5	83.9±7.2	83.7±5.7	81.6±6.0	81.0±6.8
P45-10	195-209	FS, 2, 3, 5	71.5±8.8	71.2±8.3	69.3±7.3	68.8±7.9

Thus, the utilization of antibodies that are specific to the aar 23-40 and/or 140-196 regions in cTnI assays might represent a good compromise that could help to minimize the negative influence of both protein degradation and interference of autoantibodies on precise cTnI measurements by immunoassays.

Conclusions

1. Intact cTnI and its 11 major fragments were detected in the blood of AMI patients.
2. The ratio of the fragments in blood did not show significant changes in the period of 1 - 36 hours following AMI.
3. Antibodies that are specific to the epitopes which include aar 23-36 and 126-196 of cTnI could be suggested for the assay development because they:
   - Recognize ≥80% of cTnI in the blood samples of patients within the first 36 hours following AMI
   - Are almost insensitive to the autoantibody interference

[1] – Katrukha AG et al. 1998, ClinChem 44(12), c. 2433-2440
[2] – Eriksson S et al. 2003, ClinBiochem 37, 472– 480