Full-Size Cardiac Troponin I and Its Proteolytic Fragments in Blood of Patients with Acute Myocardial Infarction: Antibody Selection for Assay Development

Ivan A. Katrukha,1,2* Alexander E. Kogan,1,3 Alexandra V. Vylegzhanina,1 Alexey V. Kharitonov,2 Natalia N. Tamm,1,3 Vladimir L. Filatov,1,3 Anastasia V. Bereznikova,1,3 Ekaterina V. Koshkina,4 and Alexey G. Katrukha1,2

BACKGROUND: In the blood of patients with acute myocardial infarction (AMI), cardiac troponin I (cTnI) presents as an intact molecule with a repertoire of proteolytic fragments. The degradation of cTnI might negatively influence its precise immunodetection. In this study we identified cTnI fragments and calculated their ratio in the blood of patients at different times after AMI to discriminate the most stable part(s) of cTnI.

METHODS: Serial serum samples were collected from AMI patients within 1 to 36 h after the onset of chest pain both before and after stenting. cTnI and its fragments were immunoextracted from serum samples and analyzed by Western blotting with monoclonal antibodies (mAbs) specific to the different epitopes of cTnI and by 2 inhouse immunoassays specific to the central and terminal portions of cTnI.

RESULTS: Intact cTnI and its 11 major fragments were detected in blood of AMI patients. The ratio of the fragments in serial samples did not show large changes in the period 1–36 h after AMI. mAbs specific to the epitopes located approximately between amino acid residues (aar) 34 and 126 stained all extracted cTnI. mAbs specific to aar 23–36 and 126–196 recognized approximately 80% to 90% (by abundance) of cTnI.

CONCLUSIONS: In addition to mAbs specific to the central part of cTnI (approximately aar 34–126), antibodies specific to the adjacent epitopes (approximately aar 23–36 and 126–196) could be used in assays because they recognize ≥80% of cTnI in patients’ blood samples within the first 36 h after AMI.

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Measurement of cardiac troponin I (cTnI)5 in blood samples of patients by immunoassay is one of the most reliable methods for the diagnosis of acute myocardial infarction (AMI) (1). However, even current methods of cTnI immunodetection might have shortcomings that influence the precise determination of the blood concentrations of cTnI. Among the factors that influence cTnI measurements is the degradation of cTnI. One of the first in vitro studies performed in necrotic cardiac tissue demonstrated the appearance of several cTnI proteolytic fragments with apparent molecular masses of 14–28 kDa (intact cTnI exhibiting an apparent molecular mass of approximately 29 kDa) (2). The region located between amino acid residues (aar) circa 30–110 of cTnI was characterized as the most stable part of the molecule. Currently, the majority of diagnostic systems utilize antibodies with epitopes located within the central part of cTnI (3).

Later studies revealed the presence of up to 11 cTnI fragments in serum samples of patients with ST-elevation myocardial infarction (4), patients with hypertrophic obstructive cardiomyopathy undergoing percutaneous septal ablation (5), or patients with presumed myocardial necrosis caused by acute ischemic stroke (6). Although these fragments have been extensively studied, their borders have not been characterized. Nevertheless, such information is crucial for the proper selection of antibodies to be used in cTnI assays. In this study, we determined the composition

1 HyTest Ltd., Turku, Finland; 2 Department of Biochemistry, School of Biology, Moscow State University, Moscow, Russia; 3 Department of Bioorganic Chemistry, School of Biology, Moscow State University, Moscow, Russia; 4 67th City Hospital, Moscow, Russia.
* Address correspondence to this author at: HyTest, Intelligate 1, 6th floor, Joukahaisenkatu 6, 20520 Turku, Finland. Fax +358-2-512-0909; e-mail ivan.katrukha@hytest.fi.
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1* Nonstandard abbreviations: cTnI, human cardiac troponin I; AMI, acute myocardial infarction; mAb, monoclonal antibody; recTnI, recombinant human cardiac TnI; TnT, troponin T; TnC, troponin C.
and the ratio of cTnI fragments present in the blood of AMI patients before reperfusion was performed and at different time points after stenting to determine the most stable part(s) of cTnI. The results of the current study suggest a new paradigm for the selection of antibodies for the new generation of cTnI immunoassays.

**Materials and Methods**

**REAGENTS**

If not stated otherwise, all chemicals were purchased from Sigma-Aldrich. A panel of anticTnI monoclonal antibodies (mAbs), including p4-14G5 (specific to the aar 1–15 of cTnI), M18 (aar 18–28), 4C2 (aar 23–29), 19C7 (aar 41–49), 560 (aar 83–93), 16A11 (aar 86–90), 8E10 (aar 86–90), 84 (aar 117–126), 441 (aar 148–158), 625 (aar 169–178), 472 (aar 182–191), MF4 (aar 190–196), and p45-10 (aar 195–209), their conjugates with horseradish peroxidase (HRP) and stable Eu$^{3+}$ chelate, recombinant human cardiac TnI (recTnI), and ternary native human cardiac troponin complex (ITC) were from HyTest.

**BLOOD SAMPLES**

All patients selected for this study had an ST-elevation myocardial infarction followed by coronary angiography and stenting. The diagnosis of AMI was confirmed by ECG and measurements of cTnT concentrations in patient blood samples by a TropT Quantitative assay on a Cobas h232 system (Roche). Serum samples were collected by venipuncture with a standard technique (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue6) 1–36 h after the onset of chest pain at 5 time periods: first, before stenting was performed (approximately 1–4 h after the onset of chest pain), and 4 more periods after stenting (2–7, 7–12, 12–23, and 23–36 h after AMI onset). All patients received clopidogrel bisulfate (Plavix or Plagril, 300 mg, 2 times: at admission and 5.2.1 software (Bio-Rad).

**IMMUNOEXTRACTION OF CTNI**

Affinity matrix used for cTnI immunoextraction used 13 anticTnI mAbs (p4-14G5, M18, 4C2, 19C7, 560, 16A11, 8E10, 84, 441, 625, 472, MF4, and p45-10) specific to the different epitopes that cover almost the entire cTnI sequence. Each mAb was separately immobilized on Sepharose CL-4B (GE Healthcare) at the final concentration of 5 g/L with a conventional BrCN method (8), then equal volumes of the individual affinity matrices were mixed together. Further immunoprecipitation was performed as described in (9). Our preliminary experiments showed that the applied immunoprecipitation method did not affect the integrity of cTnI; affinity matrix was capable of extracting all forms (free cTnI and cTnI as a part of binary TnI-TnC and ternary ITC complexes), and all fragments of cTnI that could be detected in Western blotting and the recovery of cTnI after extraction exceeded 80% (for details, see the online Data Supplement).

**WESTERN BLOTTING AND ENHANCED CHEMILUMINESCENCE (ECL) DETECTION**

Proteins were separated with a 15%–22.5% gradient SDS-PAGE according to Laemmli (10) under reducing conditions by a Hoefer SE280 electrophoresis unit (gel size 10 × 12 cm), blotted onto an Amersham HyBond-ECL nitrocellulose membrane (GE Healthcare), immunostained with anticTnI mAbs conjugated with HRP, and visualized with SuperSignal West Fermo Maximum Sensitivity Substrate (Thermo Scientific) on the ChemiDoc Touch Imaging System (Bio-Rad) (for details, see the online Data Supplement). The apparent molecular masses of the separated proteins were determined with an LMW-SDS Marker Kit (GE Healthcare).

To determine the approximate borders of cTnI fragments, proteins immunoprecipitated from serum samples of AMI patients (n = 9) were applied on 1-well electrophoresis gels and after separation were blotted onto a nitrocellulose membrane. Then, the membrane was cut into strips, and each strip was immunostained with 1 of the 15 mAbs specific to the different epitopes of cTnI.

**ECL IMAGE QUANTIFICATION**

Image quantification was performed with ImageLab 5.2.1 software (Bio-Rad).

To calculate the ratio of the individual band, its chemiluminescence intensity was divided by the integral intensity of all bands corresponding to cTnI and its fragments in the lane and expressed as percentages. Control experiments with BrCN cleavage-derived fragment of recTnI (aar 1–154) showed that mAb 560 stained fragmented cTnI with the same efficiency as the full-size molecule (see the online Data Supplement).
STUDYING OF CTNI DEGRADATION BY MEANS OF FIA

The concentration of cTnI in serial serum samples of AMI patients was measured by 2 inhouse FIA: 19C7-560 [both mAbs recognize central, most stable part of the molecule: aar 41–49 and aar 83–93; in the current study this assay was also used as a “gold standard” for determination of cTnI concentration] and MF4-4C2 [mAbs recognize C-terminal (aar 190–196) and N-terminal (aar 23–29) parts of the molecule]. In both assays, the detection mAb was labeled with a stable Eu3+/H11001 chelate (for details, see the online Data Supplement).

To estimate the dynamics of the degradation of cTnI terminal regions in the samples taken at different times, the ratio of concentrations measured by these 2 assays was calculated.

QUANTIFICATION OF THE AMOUNT OF CTNI THAT COULD BE DETECTED BY DIFFERENT MABS IN THE BLOOD OF AMI PATIENTS

To quantify the amount of cTnI that could be detected by mAbs specific to the different portions 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 after staining by mAb 560. Thus, knowing the set of fragments that each mAb is capable of staining and the relative abundance of each fragment, the fraction of cTnI that each mAb can interact with was calculated.

STATISTICAL ANALYSIS

If not stated otherwise, the data are presented as median values and whiskers represent 25th and 75th percentiles.

Mann–Whitney U-test was used to determine the statistically significant difference between the values obtained after analysis of the samples that were taken before and after stenting. Kruskal–Wallis H-test was used to determine the statistically significant difference between the values obtained after measurement of the samples taken at 4 different time points after stenting. A value of \( P < 0.05 \) was considered statistically significant. Origin 7.5 software (OriginLab) was used to perform the statistical analysis.

Results

WESTERN BLOTTING ANALYSIS OF CTNI AND ITS FRAGMENTS EXTRACTED FROM SERUM OF AMI PATIENTS

The results of Western blotting analysis of cTnI immunoextracted from serum samples of 26 AMI patients revealed that (a) all patients had a similar set of 12 bands that corresponded to the full-size cTnI molecule and its 11 fragments and (b) the number of fragments remained the same, independent of the time passed after AMI (Fig. 1).

Immunostaining of the fragments by 15 mAbs specific to the different epitopes of cTnI revealed that in blood of AMI patients, cTnI is presented by the forms truncated from both the N- and C-terminal parts of the molecule (Fig. 2). From the N-terminus, 3 major sites of proteolysis could be allocated: the first, between epitopes of the mAb 909 (epitope aar 18–22) and 4C2 (aar 23–29) (Fragments 2, 4); the second, between epitopes of mAb 4C2 and M155 (aar 26–35) (Fragments 3, 5); the third, between epitopes of mAb M155 and 10F4 (aar 34–37) (Fragments 5 and 7). At the C-terminal portion of the molecule, we observed multiple cleavage sites. Antibody 84 stained all visible cTnI fragments bordering the most stable region of cTnI from the C-terminus close to aar 126 (Fragment 11). Therefore, the most stable region of the cTnI molecule present in the blood of AMI patients can be defined as a fragment located approximately between aar 34–126.
full-size cTnI represented approximately 40% of all cTnI detected by mAb 560, whereas the 2 shortest detected fragments (fragments 10 and 11, approximately aar 23–146 and aar 23–126, respectively) represented only about 10% of all cTnI (Fig. 3).

Western blotting analysis of cTnI and its fragments from serum samples of AMI patients that were taken before and 1–2 h after stenting (n = 10) revealed that percutaneous intervention did not significantly affect either the number or the ratio of cTnI fragments (Mann–Whitney analysis P > 0.05 for all detected fragments or fragment groups) (Fig. 3, A and B). The analysis of serial serum samples taken at 4 periods after stenting has shown that the changes in the ratio of full-size cTnI and its fragments within the first 36 h after AMI were also statistically insignificant (Kruskal–Wallis analysis P > 0.05 for all detected fragments or fragment groups) (Fig. 3, C and D).

IMMUNOASSAY ANALYSIS OF CHANGES OF CTNI FRAGMENTS’ ABUNDANCE IN SERUM SAMPLES OF AMI PATIENTS OVER TIME
To verify the results of the previous experiment by an alternative method, we determined the concentration of cTnI in serial serum samples by 2 inhouse immunoassays, MF4-4C2 and 19C7-560, and calculated the ratio of measured concentrations (MF4-4C2/19C7-560). If the terminal regions of cTnI were cleaved off over the study timeframe, we would have seen a considerable decrease in the ratio; if the terminal regions were not cleaved off, the ratio would not decrease.

In good agreement with the results of Western blotting studies, no significant difference between the ratios of concentrations measured by the 2 assays in the samples taken prior to and after stenting (n = 10) was observed [median values of the ratio were 0.9 (25th and 75th percentiles of 0.3–1.4) and 1.3 (0.8–1.6), respectively; Mann–Whitney test result P = 0.39]. This affirms the conclusion that stenting did not change the composition and ratio of cTnI fragments present in the blood of AMI patients to a large extent.

While testing the serial samples taken after stenting (n = 64), we have observed only a 2-fold fall of the ratio of measured concentrations within the 36 h after AMI onset (P < 0.05 by Kruskal–Wallis test) (Fig. 4). This corroborates with the results of Western blotting analysis and indicates that the composition of cTnI fragments that are present in patient’s blood does not change very much within the first 36 h after AMI.

QUANTIFICATION OF THE AMOUNT OF CTNI THAT COULD BE DETECTED BY DIFFERENT MABS IN THE BLOOD OF AMI PATIENTS
Quantification of the amount of cTnI that could be detected by different mAbs in the blood of AMI patients showed that despite having a smaller number of cleavage sites, the very N-terminal portion of cTnI is more prone to degradation than the C-terminal portion (Table 1):
Fig. 3. Change of cTnI fragment ratios in serum samples of AMI patients in time.

A: A representative Western blotting demonstrating the abundance of the full-size cTnI and its fragments in samples before (B) and approximately 1 h after (A) stenting. Time after the onset of chest pain and cTnI concentration in the initial samples are indicated below the lane number. Staining with the HRP-labeled mAb 560.
1: cTnI standard (ternary ITC complex);
2: Negative control (immunoextraction of proteins from NHS);
3–8: cTnI immunoextracted from serum samples of 3 representative AMI patients (Pat 1–3).
To get a clearer picture, different amounts of the samples were applied on the well and different times of exposure were used for each patient.

B: Changes of the ratio of different cTnI fragments from serum samples that were taken before and after stenting (median values for 10 AMI patients). Whiskers represent the 25th and 75th percentiles.

C: Representative Western blotting demonstrating the abundance of full-size cTnI and its fragments in serial blood samples of 3 AMI patients taken at 4 time points after stenting. Time after the onset of chest pain and cTnI concentration in the initial samples are indicated below the lane number. Staining with HRP-labeled mAb 560.
1: cTnI standard (recTnI);
2: Negative control (immunoextraction of proteins from NHS);
3–14: cTnI immunoextracted from serial serum samples of 3 representative AMI patients.
To get a clearer picture, different amounts of the samples were applied on the well and different times of exposure were used for each patient.

D: Changes of the ratio of different cTnI fragments from serial serum samples that were taken in 4 periods after stenting (median values for 26 AMI patients). Whiskers are shown for the ratio of the 3 most abundant bands (full-size molecule, fragment 2, and fragments 10 and 11) and represent the 25th and 75th percentiles.
mAbs 9F6 and 909, specific to the very N-terminal fragment of cTnI (epitopes aar 11–15 and aar 18–22, respectively), recognized only approximately 55% of the extracted cTnI, whereas mAb p45-10, specific to the very C-terminus (aar 195–209), stained approximately 70% of all detected protein.

mAb 4C2 (aar 23–29) antibodies, specific to the N-terminal and central region of cTnI, recognized ≥90% of all detectable cTnI and its fragments present in blood. From the C-terminus, mAb MF4 (aar 190–196) interacted with approximately 80% of all extracted cTnI. Therefore, mAbs with epitopes falling within aar 23–196

![Graph showing cTnI degradation as measured by FIA.](image)

**Fig. 4.** 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 an 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). Samples were taken in 4 periods after stenting. Whiskers represent the 25th and 75th percentiles for the median.

**Table 1.** Relative amount of cTnI stained by different mAbs in serial serum samples of AMI patients (n = 26).a

<table>
<thead>
<tr>
<th>mAb</th>
<th>Epitope (aar)</th>
<th>Fragments that comprise the epitope</th>
<th>Amount of cTnI (%) stained by mAb 560</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FS, 1, 6, 8, 9</td>
<td>6 h</td>
</tr>
<tr>
<td>9F6</td>
<td>1–15</td>
<td>58.9 ± 12.3 56.9 ± 10.8 54.2 ± 10.1</td>
<td>53.4 ± 12.8</td>
</tr>
<tr>
<td>909</td>
<td>18–22</td>
<td>58.9 ± 12.3 56.9 ± 10.8 54.2 ± 10.1</td>
<td>53.4 ± 12.8</td>
</tr>
<tr>
<td>4C2</td>
<td>23–29</td>
<td>94.0 ± 2.2 93.8 ± 2.5 93.9 ± 2.6</td>
<td>93.5 ± 3.1</td>
</tr>
<tr>
<td>M155</td>
<td>26–35</td>
<td>95.5 ± 2.2 95.5 ± 2.0 95.5 ± 1.6</td>
<td>95.7 ± 1.6</td>
</tr>
<tr>
<td>MF4</td>
<td>190–196</td>
<td>83.9 ± 7.2 83.7 ± 5.7 81.6 ± 6.0</td>
<td>81.0 ± 6.8</td>
</tr>
<tr>
<td>P45-10</td>
<td>195–209</td>
<td>71.5 ± 8.8 71.2 ± 8.3 69.3 ± 7.3</td>
<td>68.8 ± 7.9</td>
</tr>
<tr>
<td>441</td>
<td>148–158</td>
<td>86.0 ± 6.6 85.9 ± 5.0 83.8 ± 5.7</td>
<td>83.0 ± 6.6</td>
</tr>
<tr>
<td>625</td>
<td>169–178</td>
<td>86.0 ± 6.6 85.9 ± 5.0 83.8 ± 5.7</td>
<td>83.0 ± 6.6</td>
</tr>
<tr>
<td>C5</td>
<td>186–192</td>
<td>85.5 ± 6.5 85.3 ± 5.1 83.1 ± 5.7</td>
<td>82.5 ± 6.3</td>
</tr>
<tr>
<td>M46</td>
<td>130–145</td>
<td>95.4 ± 2.5 95.3 ± 1.8 94.2 ± 2.4</td>
<td>93.8 ± 2.5</td>
</tr>
<tr>
<td>10F4,</td>
<td>19C7, 560,</td>
<td>954 ± 25 953 ± 18 942 ± 24 938 ± 25</td>
<td></td>
</tr>
<tr>
<td>16A11,</td>
<td>84</td>
<td>100 100 100 100</td>
<td></td>
</tr>
</tbody>
</table>

a Ratio of cTnI (full-size molecule (FS) and fragments) that could be recognized by the specific antibody to the total cTnI (full-size cTnI and its fragments that are detected by the reference mAb 560, which recognizes all extracted peptides). Mean% ± SD.
recognized >80% of all cTnI detected in patients’ samples taken within the first 36 h after AMI.

Discussion

In an immunoassay, the utilization of mAbs that are specific to the cleavable region of the analyte might lead to serious underestimation of the analyte’s concentration in the sample, with potential for misdiagnosis in some patients or patient groups. Early studies showed that the N- and C-terminal portions of cTnI (approximately aar 1–30 and 110–210) are prone to degradation, suggesting obvious limitations for the utilization of the antibodies specific to these unstable fragments (11). However, in several studies, it was shown that most of the antibodies that recognize “stable” central epitopes are sensitive to the autoantibodies present in the blood of some patients (12–15). Such autoantibodies that interfere with the assay’s mAbs, can negatively influence cTnI measurements, and in some cases (early samples or small-size AMI) this interference might lead to false-negative results. Obviously, these 2 factors that influence the precise measurements of cTnI — cTnI degradation and autoantibodies — for many years puzzled the researchers who selected antibodies for cTnI assays. The goal of the current study was to analyze the composition and prevalence of the proteolytic fragments of cTnI in the blood of AMI patients to understand whether mAbs specific to the regions other than aar 30–110 of cTnI could be used in cTnI assays without the risk of significant underestimation of the analytes’ concentration or obtaining false-negative results.

Our Western blotting studies of cTnI in the serum samples of AMI patients revealed that blood samples collected from different patients and at different times had similar spectra of cTnI fragments. By using mAbs with known epitope specificities, we were able to identify and establish the borders of 11 proteolytic fragments of cTnI cleaved from the N- or C-terminal portions of the molecule or both. The smallest detected fragment of cTnI in the blood is bordered by the epitopes of mAbs 4C2 (aar 23–26) from the N-terminus and 84 (aar 117–126) from the C-terminus (Fig. 2). Peptides 5 and 7, which are separated from the N-terminus by the epitope of mAb 10F4 (aar 34–37), restrict the most stable region to approximately aar 34–126. These data obtained for cTnI extracted from blood are in good agreement with our previously published in vitro studies, in which we analyzed the cTnI degradation in cardiac tissue (2). The antibodies specific to aar 34–126 recognize all immunoreactive cTnI forms extracted from the blood of AMI patients.

To ensure the reliable detection of the low-abundance cTnI fragments by means of Western blotting, we analyzed the samples with relatively high cTnI concentrations (approximately 1–200 ng/mL). Despite such a wide range of cTnI concentrations, we have not observed any major difference in the distribution of cTnI fragments in the analyzed samples. We presume that in the samples with concentrations lower that 1 ng/mL the ratio of cTnI fragments also should not show major differences.

We were not able to detect any fragments smaller than 14 kDa even by using Tricine gel electrophoresis that is designed to separate peptides of a mass as low as 1–2 kDa (data not shown). We presume that the cTnI fragments smaller than 30–130 aar might no longer be capable of binding to cTnC (which protects cTnI from protease-mediated degradation (2)) and, consequently, are either rapidly degraded in the necrotic tissue and/or blood to very small pieces that are not recognized by mAbs and/or are eliminated from the circulation right after their release to the blood flow.

In early in vitro studies of cTnI degradation in cardiac tissue (2), we observed a gradual time-dependent degradation of the full-size molecule and its larger fragments into small-size fragments with molecular masses of 14–18 kDa. Later the degradation of cTnI was observed by McDonough et al. (16) in the myocardial biopsy samples of bypass patients and by Medsen et al. (4) in serum samples of AMI patients taken at different times after reperfusion. On the basis of these findings, for a long time it was thought that cTnI is mainly present as the full-size molecule in early blood samples, that is gradually replaced by large size fragments, and then finally by fragments that encompass aar 30–110 or even smaller.

Unexpectedly and in contrast to the in vitro studies, in this study we did not observe major changes in the ratio of the full-size cTnI molecule and its fragments in the blood samples taken before and after stenting and within 2–36 h after the onset of AMI by 2 independent methods: Western blotting and FIA. The ratio of the full-size cTnI and its fragments in blood remained more or less unchanged over time.

On the basis of these observations, we suggest that the degradation of cTnI occurs mainly in the necrotic myocardium but not in the blood of AMI patients. Cell death in the ischemic myocardium is a continuous process, and it appears that as soon as myocytes and their contractile apparatus reach some threshold level of degradation, cTnI and its fragments are washed away from myofilaments into the bloodstream. Because of this equilibrium of degradation/washing away, the ratio of full-size cTnI and its fragments in blood remains almost unchanged in time. Early stenting restores the blood flow in the ischemic part of the muscle and prevents further necrosis of cardiac tissue and more profound degradation of cTnI. However, we cannot exclude the possibility that if stenting was performed at later times, it might lead to the release of a much larger amount of the smaller fragments...
of cTnI from the “core” necrotic area that was restricted from blood before the reperfusion. We also need to note that in this study we have analyzed the samples of patients with AMI only, so we cannot be sure that the distribution of cTnI fragments would be the same in the samples of patients with other diseases that affect myocardium or cTnI clearance from the blood flow (i.e., chronic heart failure, chest trauma, toxic injury of myocardium, chronic kidney disease).

Our results contradict the results of the study by Madsen et al. (4), in which only the full-size cTnI was detected in the samples taken during the first 90 min following reperfusion, with the gradual appearance of proteolytic fragments of cTnI in the first 90–120 min after stenting. This was accompanied by a rise of the cTnI concentration in the samples from <1 to >100 ng/mL. But after that, no substantial change in the ratio of fragments was observed. We may presume that the absence of proteolytic fragments in the samples taken during the first 90 min after stenting might be explained by the lack of sensitivity of the detection system used in the cited study, which was not capable of staining cTnI fragments because of the low anlyte concentration in the early samples. The results of the later work by Madsen et al. (5) generally confirm the results presented in this study, as these authors observed considerable amounts of the full-size cTnI and its high molecular weight fragments even 50 h after percutaneous septal ablation.

The finding that antibodies with epitopes located between amino acid residues 23–196 recognize >80% of cTnI in a patient’s sample and that this ratio does not change very much in the first 36 h after AMI is very important from a practical point of view. Although the fragment from aar 30–110, which was suggested earlier as a target for the assay antibodies, is rather big (about 30% of the protein), even these authors observed considerable amounts of the full-size cTnI and its high molecular weight fragments even 50 h after percutaneous septal ablation.

In this study, we demonstrated that cTnI is present in the blood of AMI patients as a full-size molecule and 11 proteolytic fragments. The ratio of full-size cTnI to its fragments does not change very much in the period of 1–36 h after the onset of AMI. The most stable part of cTnI present in the patients’ blood is approximately demarcated by aar 34–126; the antibodies with the epitopes located within aar 23–196 recognize >80% of all immunoreactive cTnI. The utilization of antibodies specific to the aar 23–40 and/or 140–196 regions in cTnI might be a good compromise that could help to minimize the negative influence of protein degradation and interference of autoantibodies on precise cTnI measurements by immunoassays.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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