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Cardiac Troponin T Levels at 96 Hours Reflect Myocardial Infarct Size: A Pathoanatomical Study

Andrew Remppis[a] Philipp Ehlermann[a] Evangelos Giannitsis[a]
Tobias Greten[b] Patrick Most[a] Margit Müller-Bardorff[a] Hugo A. Katus[a]

[a]Medizinische Klinik II, Medizinische Universität zu Lübeck, Lübeck, [b]Innere Medizin/Kardiologie, Herzzentrum Lahr/Baden, Lahr, Deutschland

Key Words
Troponin T · Myocardial infarction · Infarct size · Enzyme kinetics · Animal model

Abstract
We determined the utility of single-point measurements of circulating cardiac troponin T (cTnT) for the noninvasive estimation of infarct size in 16 beagle dogs after left anterior descending artery (LAD) ligation. Pathoanatomical infarct sizes were determined by the triphenyltetrazolium chloride method and correlated with serum concentration changes of cTnT. Peak cTnT levels (14.10 ± 4.71 μg/l) were reached after 110 ± 21 h. A significant correlation was found between peak cTnT levels ($p = 0.0001$, $r = 0.83$) or cumulative cTnT levels and relative infarct size ($p = 0.0010$, $r = 0.72$). A single cTnT measurement 96 h after LAD ligation was equally predictive of infarct size ($p = 0.0010$, $r = 0.74$) as peak or cumulative cTnT levels derived from serial sampling. cTnT levels at 96 h may thus be useful for practical and cost-effective estimation of infarct size.

Introduction

Cardiac troponin T (cTnT) has been introduced into routine clinical testing for suspected myocardial cell damage. The widespread use of this marker is mostly due to its higher sensitivity for myocardial necrosis as compared to creatine kinase MB (CK-MB) [1]. cTnT furthermore allows the identification of a high-risk subgroup of patients with unstable angina pectoris that cannot be classified by cardiac enzymes [2–4]. These findings and the markedly higher specifity of cTnT versus CK-MB in patients with skeletal muscle injury have put into doubt the role of CK-MB as the golden standard in the biochemical diagnosis of acute myocardial infarction.

Noninvasive estimation of infarct size by measuring the blood levels of CK-MB is routinely employed despite its known limitations. In contrast to CK-MB, cTnT exists as a minor free cytosolic and a major structurally bound pool [5]. The release of the structural cTnT depends on the disintegration of the contractile apparatus. This time-consuming process continues when changes in the washout kinetics of cardiac markers by reperfusion of the infarct zone are no longer evident. Thus, the washout

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Dr. Andrew Remppis
Medizinische Klinik II, Medizinische Universität zu Lübeck
Ratzeburger Allee 160
D–23538 Lübeck (Germany)
Tel. +49 451 500 2500, Fax +49 451 500 6437, E-Mail remppis@medinf.mu-luebeck.de

kinetics of the structural cTnT pool are not affected by thrombolytic therapy [6]. These characteristics correspond to the release kinetics of myosin light chains and myosin heavy chains. The blood levels of these markers reflect pathoanatomical infarct size [7].

Only a few studies have been published on the utility of cTnT for the estimation of infarct size. In a well-conducted experimental study, Ricchiuti et al. [8] demonstrated the relationship between cTnT cumulative release or peak value and histological infarct size and tissue depletion of cTnT. In three clinical studies, cTnT levels were investigated in relation to clinical indicators of infarct size and found to be significantly related [9–11]. However, in clinical practice and considering the price of cTnT testing, serial analyses of cTnT levels are not feasible. Therefore, we tested the usefulness of cTnT levels determined at a fixed time point after the onset of acute myocardial infarction for a noninvasive estimation of infarct size in an experimental canine model.

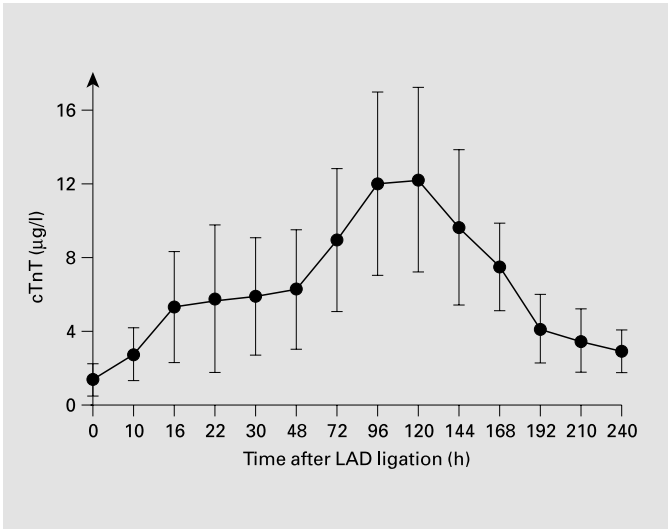

Fig. 1. cTnT time release curve. Values are given as mean and standard deviations (bars). cTnT serum levels show monophasic release kinetics with a steady increase until days 4 and 5.

Materials and Methods

Animal Preparation

The present study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) and with the guidelines of the Landesministerium für Forschung und Technik of Baden-Württemberg, Germany. This is a substudy of an investigation performed to characterize endocardial monophasic action potentials in infarcted canine ventricles. The experiments were performed according to Schmitt et al. [12]. Briefly, 20 beagle dogs (13 male and 7 female dogs) with a mean weight of 14.9 kg (12.3–16.5 kg) were intravenously anesthetized with 30 mg/kg body weight sodium phenobarbital (Nembutal®). As premedication, 0.05 mg/kg body weight N-3-dimethylaminopropyl-2-propionyl-phenothiazine (Combelen®) was applied intramuscularly. Dogs were mechanically ventilated with an N_2O/O_2 gas mixture (70/30 vol%) by a volume-controlled respirator system (Engstroem Respirator System ER 300). Lateral thoracotomy was performed by incision into the fourth intercostal space; pericardiotomy was performed along the heart axis. The left anterior descending artery (LAD) was prepared 1.5 cm distal to the first septal branch and a subtotal stenosis was produced by a snare-type polyethylene occluder. In the absence of higher-degree arrhythmias in the following 20 min, complete ligation of the vessel was induced. A central venous catheter for blood sampling was implanted subcutaneously. All animals received 0.3 mg of buprenorphine (Temgesic®) intramuscularly after the return of spontaneous breathing. As prophylaxis against postoperative infections, 1 g of ampicillin (Binotal®) was administered for 3 days. After 7–15 days, the dogs were sacrificed and the hearts explanted.

Determination of Infarct Size

After explantation, the hearts were rinsed in ice-cold saline and sectioned in transverse slices of 1 cm in width from apex to base. These sections were incubated in 2,3,5-triphenyltetrazolium chloride (TTC) and 0.2 mol/l phosphate buffer, pH 7.8, at 37 °C for 5–7 min according to Sandritter and Jestadt [13], in order to define the infarcted myocardium. The infarcted areas were dissected and weighed. Relative infarct size was calculated in relation to heart weight.

Measurement of Serum Markers

Serial blood samples were obtained immediately before the operation and 2, 4, 10, 16, 22 and 30 h thereafter, then daily until the animals were sacrificed. All biochemical analyses were performed immediately or from frozen serum samples stored at –20 °C, within 2 weeks after the samples were collected.

cTnT (reference value <0.1 µg/ml) was measured by a second-generation commercial ELISA on an ES300 analyzer (Enzymun Troponin T, Roche-Diagnostics, Mannheim, Germany) employing streptavidin-coated tubes and two monoclonal antibodies against cTnT. The lower detection limit of this assay is 0.02 µg/l. This assay exhibits no significant cross-reactivity with skeletal muscle cTnT [14], but cross-reacts with canine cTnT.

Statistical Analyses

The statistical analyses were performed with SAS (Statistical Analysis System). If not otherwise indicated, all data are expressed as mean values ± standard deviation. Cumulative cTnT release was calculated as the area under the curve for each animal. The correlations were calculated as linear regression analyses for maximum concentrations and cumulative kinetics of cTnT. Significance was considered at $p < 0.05$.

Table 1. Characteristics of the 16 animals that underwent LAD ligation

Animal No.	Body weight kg	Heart weight g	Infarct weight g	Relative infarct weight, %	Peak cTnT µg/l	Cumulative cTnT mg·h/l	96-hour cTnT µg/l
1	16.5	103.4	14.4	13.9	9.77	1,108	6.48
2	15.7	90.0	16.5	18.3	19.90	2,049	19.90
3	13.5	70.0	16.2	23.1	16.71	1,664	11.79
4	14.8	71.0	17.7	24.8	21.10	1,772	21.10
5	15.6	100.0	21.5	22.5	14.48	1,689	11.91
6	13.4	68.7	11.9	17.3	16.37	2,192	15.82
7	15.1	81.0	11.5	14.2	11.03	1,323	8.77
8	14.9	88.8	17.2	19.4	18.70	2,096	13.35
9	16.0	99.0	10.8	10.8	13.34	1,463	13.34
10	15.4	91.0	8.0	8.8	7.38	909	5.88
11	14.3	76.5	16.4	21.4	15.63	1,824	12.09
12	12.3	56.5	7.1	12.5	10.54	1,260	7.18
13	15.5	91.6	12.8	14.0	12.40	1,731	12.40
14	16.0	92.0	1.5	1.6	3.04	283	2.95
15	15.5	91.5	11.6	12.7	18.00	1,882	11.00
16	14.2	93.0	19.5	21.0	17.16	1,378	17.16
Mean ± SD	14.9 ± 1.1	85.2 ± 12.8	13.4 ± 4.9	16.0 ± 5.9	14.1 ± 4.7	1,539 ± 493	12.0 ± 4.8

Results

Twenty dogs underwent LAD ligation; 2 died during the operation, while in 2 dogs the ligation of the LAD was inappropriate. Sixteen dogs (12 male, 4 female) were therefore finally analyzed. Hearts were explanted 229.5 ± 64.6 h after coronary ligation. Absolute infarct size amounted to 13.4 ± 4.9 g, yielding a relative infarct size of 16.0 ± 5.9%. The baseline characteristics of all the dogs analyzed are given in table 1. Figure 1 shows the cTnT serum concentration curve of cTnT mean values with standard deviations. While cTnT was not detectable in serum at baseline, cTnT levels increased above the cutoff limit (0.1 µg/l) in all dogs as early as 4 h after LAD ligation. Thereafter, cTnT serum levels steadily rose to a peak level of 14.10 ± 4.71 µg/l at 110 ± 21 h (range 48–144 h).

Relative infarct size was correlated with cumulative and peak cTnT values, as well as with cTnT serum levels at each sample collection time between 4 and 168 h after LAD ligation. There was a highly significant correlation when cumulative ($r = 0.72$, $p = 0.0017$; fig. 2b) and peak ($r = 0.83$, $p = 0.0001$; fig. 2c) cTnT levels were analyzed. Analyses of cTnT levels at each single time point (table 2) revealed that a significant correlation was reached only for cTnT levels 96 h after LAD ligation ($r = 0.74$, $p = 0.0010$; fig. 2a).

Discussion

The present study compared cTnT serum levels with pathoanatomical infarct size in dogs. The chosen experimental model of myocardial ischemia results in a nonreperfused myocardial infarction with a monophasic serum TnT time release curve, as described in patients with nonreperfused myocardial infarction after thrombolytic therapy [5].

Recent studies have compared cTnT or cTnI release with other independent methods for the quantification of infarct size. Omura et al. [9] compared late serum TnT peak concentrations in 34 patients with infarct size as estimated by left ventriculography, two-dimensional echocardiography and resting 201-thallium myocardial single-photon emission computed tomography. They demonstrated an excellent, linear correlation between the extent and severity of acute myocardial infarction 4 weeks after the onset of infarction and late TnT peak concentration between the 3rd and 5th day after the onset of infarction. Tanaka et al. [10] reported a close correlation between infarct size as estimated by left ventriculography and early-peak cTnI and cTnT levels in patients with successfully reperfused acute myocardial infarction. Likewise, Wagner et al. [11] found a significant correlation between scintigraphic estimates of infarct size using single-photon emission computed tomography and cTnT peaks.

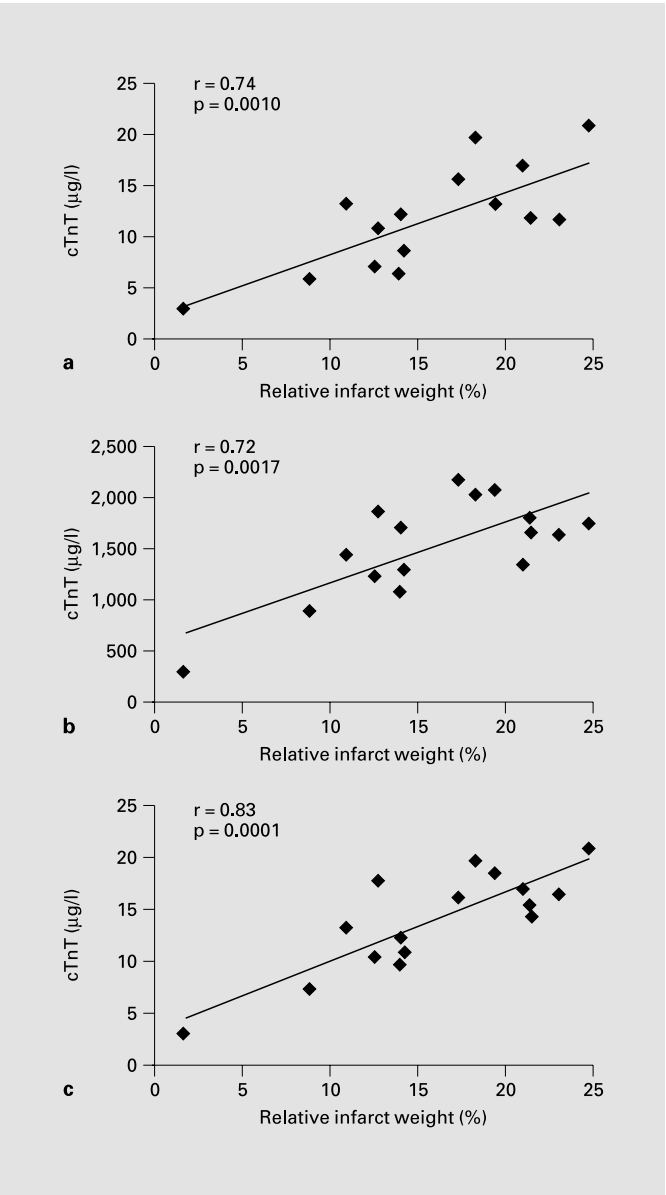

Fig. 2. Correlation of cTnT values with relative infarct weights. **a** 96-hour cTnT levels. **b** Cumulative cTnT levels. **c** Peak cTnT levels.

Table 2. Mean values ± standard deviation of cTnT levels and correlations of cTnT levels at different sampling time points with the pathoanatomical infarct size

Time, h	n	cTnT, µg/l	r	p
4	16	1.27 ± 0.84	0.022	0.9339
10	15	2.67 ± 1.42	0.229	0.4105
16	13	5.23 ± 2.98	–0.334	0.2641
22	16	5.67 ± 4.00	–0.044	0.8696
30	15	5.81 ± 3.17	0.295	0.2853
48	16	6.23 ± 3.21	0.229	0.3922
72	16	8.90 ± 3.86	0.442	0.1035
96	**16**	**11.95 ± 5.00**	**0.689**	**0.0031**
120	16	12.17 ± 5.04	0.485	0.0567
144	13	9.57 ± 4.21	0.544	0.0546
168	11	7.38 ± 2.38	0.209	0.5374
192	9	4.09 ± 1.84	0.634	0.0662
216	6	3.42 ± 1.70	0.857	0.2090
240	6	2.84 ± 1.13	0.288	0.5790
Peak cTnT			0.763	0.0006
Cumulative cTnT			0.656	0.0058

In contrast to these studies providing indirect evidence, our study now provides direct evidence that cTnT is applicable for an accurate estimation of infarct size. As a main finding, cumulative cTnT release and late-peak cTnT serum concentrations were significantly correlated with pathoanatomical infarct size. Our results are consistent with those of Ricchiuti et al. [8], who compared both tissue loss and serum release of cTnT and cTnI in the ischemic dog model. They found an excellent correlation between the tissue loss of cTnT ($r = 0.9$, $p < 0.05$), but not of cTnI ($r = 0.36$, $p < 0.54$), and the pathoanatomical infarct size as quantified by the TTC method. Serum release of cTnT was monophasic, peaking 5 days after coronary artery ligation, as in our experiments.

We additionally correlated cTnT measurements at each time point with infarct sizes in order to test the applicability of one-point measurements for infarct size determination. Among different single one-point measurements cTnT, the levels 96 h after the onset of ischemia showed a close correlation with infarct size ($r = 0.74$, $p < 0.005$) that was comparable to cumulative cTnT levels ($r = 0.72$, $p < 0.002$) and late cTnT peak levels ($r = 0.83$, $p = 0.0001$). cTnT levels on days 3 and 5, however, did not show a significant correlation, which may be due to a smaller infarct size in our experimental setting as compared to Omura et al. [9].

Single cTnT measurements 96 h after the onset of pain may provide a practical and cost-effective algorithm for the noninvasive estimation of infarct size in patients. This approach appears to be more useful than analyzing cumulative cTnT release, due to a possible incomplete recovery of cTnT [15] and the requirement of repetitive sampling. The same limitation applies to the assessment of late cTnT peak levels, which also depend on close cTnT monitoring.

It appears reasonable to extrapolate our results to the human setting of myocardial infarction, since the release kinetics of cTnT after coronary artery occlusion in canines and nonreperfused myocardial infarction in humans are similar [16, 17]. Furthermore, our results obtained in nonreperfused myocardial infarction might just as well apply to patients with reperfused myocardial infarction, since thrombolytic therapy obviously does not influence the release characteristics of the structurally bound cTnT pool [5, 9].

While the value of early cTnT measurements for risk stratification of patients with acute myocardial infarction and unstable angina has been documented in many randomized and prospective trials, the prognostic power of late cTnT levels in patients with confirmed myocardial infarction has not yet been evaluated. Therefore, further prospective studies are warranted to clarify the role of late-peak troponin levels for risk assessment and estimation of infarct size in patients with confirmed acute myocardial infarction.

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