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Variation of Cardiac Troponin I and T Measured with Sensitive Assays in Emergency Department Patients with Noncardiac Chest Pain

Volkher Scharnhorst,^{1*} Krisztina Krasznai,² Marcel van 't Veer,² and Rolf H. Michels²

BACKGROUND: New-generation high-sensitivity assays for cardiac troponin have lower detection limits and less imprecision than earlier assays. Reference 99th-percentile cutoff values for these new assays are also lower, leading to higher frequencies of positive test results. When cardiac troponin concentrations are minimally increased, serial testing allows discrimination of myocardial infarction from other causes of increased cardiac troponin. We assessed various measures of short-term variation, including absolute concentration changes, reference change values (RCVs), and indices of individuality (II) for 2 cardiac troponin assays in emergency department (ED) patients.

METHODS: We collected blood from patients presenting with cardiac chest pain upon arrival in the ED and 2, 6, and 12 h later. Cardiac troponin was measured with the high-sensitivity cardiac troponin T (hs-cTnT) assay (Roche Diagnostics) and a sensitive cTnI assay (Siemens Diagnostics). Cardiac troponin results from 67 patients without acute coronary syndrome or stable angina were used in calculating absolute changes in cardiac troponin, RCVs, and II.

RESULTS: The 95th percentiles for absolute change in cardiac troponin were 8.3 ng/L for hs-cTnT and 28 ng/L for cTnI. Within-individual and total CVs were 11% and 14% for hs-cTnT and 18% and 21% for cTnI, respectively. RCVs were 38% (hs-cTnT) and 57% (cTnI). The corresponding log-normal RCVs were +46%/–32% for hs-cTnT and +76%/–43% for cTnI. II values were 0.31 (cTnI) and 0.12 (hs-cTnT).

CONCLUSIONS: The short-term variations and IIs of cardiac troponin were low in ED patients free of ischemic myocardial necrosis. The detection of cardiac troponin variation exceeding reference thresholds can help to identify ED patients with acute myocardial necrosis

whereas variation within these limits renders acute coronary syndrome unlikely.

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Myocardial infarction (MI)³ is defined by the Joint Task Force for the Redefinition of MI as a rise or fall in cardiac troponin in the appropriate clinical background with at least 1 measurement of cardiac troponin exceeding the 99th percentile of a reference population (1). Currently, sensitive cardiac troponin assays can measure cardiac troponin concentrations well within the reference interval (2, 3, 4). Depending on the reference population, different 99th percentiles are found with the use of the same assay (5). For example, 99th percentiles ranging from 40 to 80 ng/L have been described for the TnI-ultra troponin I assay from Siemens Diagnostics (4, 6, 7), and from 13.5 to 16 ng/L for the high-sensitivity cardiac troponin T (hs-cTnT) assay from Roche (8, 9). In the future, more sensitive tests that can measure cardiac troponin concentration in any individual may enter clinical practice. Thus, the 99th percentile will depend strongly on the reference group chosen. Therefore, it becomes mandatory to discriminate bona fide MI from physiological or other pathological elevations within patient groups. Furthermore, because sensitive cardiac troponin assays reliably measure slight increases in cardiac troponin, it is essential to discriminate acute myocardial damage as a result of ischemia from other causes of myocardial necrosis (10) that lead to chronic increases in cardiac troponin concentrations. Decision thresholds need to be established with regard to what change in cardiac troponin concentration is needed minimally to satisfy the criterion “rise or fall in cardiac troponin” for detection of MI. The 2007 consensus statement (1) suggests a change of 20%; however, this percentage is arbitrary

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³ Nonstandard abbreviations: MI, myocardial infarction; hs-cTnT, high-sensitivity cardiac troponin T; NSTEMI, non-ST-elevation MI; RCVs, reference change values; II, index of individuality; ED, emergency department; ACS, acute coronary syndrome; ECG, electrocardiogram; UAP, unstable angina pectoris; CV_i, within-individual biological imprecision; CV_t, coefficient of the total imprecision.

and not evidence based. Therefore, the 2011 guideline of the European Society of Cardiology on diagnosis and treatment of non-ST-elevation MI (NSTEMI) states that a definition of clinically significant change in cardiac troponin needs to be established for each assay (10).

Recently reported studies have addressed variability of cardiac troponin as measured with high-sensitivity assays (11–13). In these investigations small numbers of healthy volunteers were used to establish reference change values (RCVs) and the index of individuality (II) (the intraindividual variation in relation to the interindividual variation) (11) of cardiac troponin over hours and weeks. A relative change of cardiac troponin concentration that exceeds the RCV is taken as clinically significant, i.e., signifying acute myocardial necrosis. Reichlin et al. (14) used another approach and tested the utility of relative and absolute cardiac troponin changes in the early detection of acute myocardial necrosis.

In this study, we investigated the short-term variation of cardiac troponin, measured with a sensitive cTnI and an hs-cTnT assay, in patients who present with cardiac chest pain to the emergency department (ED) without acute coronary syndrome (ACS). In conjunction with the 99th percentile of a reference population, the assay-specific, relative, and absolute change values described here can be applied in ruling out acute myocardial necrosis.

Materials and Methods

We used patient plasma from a previous study regarding the value of the cTnI assay (Siemens Medical Solutions Diagnostics) to detect MI in the ED (15). That study was conducted according to the declaration of Helsinki and approved by the Medical Ethical commission of Catharina Hospital according to Dutch law. The protocol was registered and published by the Dutch Central Committee on Research involving Human Subjects (CCMO; www.ccmo-online.nl) as trial number NL16413.060.07. In brief, every patient entering the ED with chest pain and an electrocardiogram (ECG) without ST-segment elevation was eligible for inclusion after signing informed consent. When the patients arrived at the hospital blood was drawn and cTnI concentrations were analyzed on an Advia Centaur immunochemistry analyzer (Siemens Medical Solutions Diagnostics, Breda, the Netherlands) with the TnI-Ultra troponin assay upon arrival at the hospital (t_0) and 2 (t_2), 6 (t_6), and 12 (t_{12}) h later. Aliquots of heparin plasma were frozen at all time points and stored at -80°C within 4 h after venipuncture. During the observation period of 12 h patients were monitored by a 12-lead ECG. If a new episode of chest pain oc-

curred, a 12-lead ECG was obtained and compared with a tracing obtained when symptoms had resolved spontaneously or after administration of nitrates. When deemed necessary, an ECG was recorded to assess left ventricle function and to eliminate other cardiovascular causes of chest pain.

After an observation period, a final clinical diagnosis in each patient was made by the attending cardiologist. Patients were divided into 4 groups: ACS-STEMI (persistent ST-wave elevation on ECG and cTnI >100 ng/L); ACS-NSTEMI (dynamic ECG changes without persistent ST elevation in combination with cTnI >100 ng/L); ACS with unstable angina pectoris (UAP) (ACS-UAP; dynamic ECG changes with cTnI ≤ 100 ng/L); and absence of acute myocardial necrosis (no ECG changes, cTnI <60 ng/L). For the hs-cTnT measurements, plasma was thawed and hs-cTnT was measured with the hs-cTnT assay on a Cobas e601 immunochemistry analyzer (Roche Diagnostics, Almere, the Netherlands). Test characteristics of the hsTnT and cTnI-ultra assays are described in (8) and (6), respectively. In our laboratory, the CVs obtained for the lowest level of QC material were 4.9% for hsTnT (at 24 ng/L) and 9.1% for TnI (at 58 ng/L).

DATA ANALYSIS

All patients without myocardial necrosis, i.e., excluding ACS-STEMI, ACS-NSTEMI, ACS-UAP and 2 patients with stable angina and cTnI concentrations >60 ng/L, were eligible for analysis.

To obtain absolute, intraindividual variation in cardiac troponin within 12 h (overall change), the greatest difference between cardiac troponin measurements was calculated per patient by subtracting the lowest from the highest concentration. We used 251 hs-cTnT and 266 cTnI results from 67 patients with cTnI and hs-cTnT measurements from at least 3 time points to calculate the greatest intraindividual change in concentration (100th percentile), the 99th, 97.5th, and 95th percentile changes. The absolute changes in cardiac troponin within 2, 4, 6, and 12 h were obtained by calculating the percentiles of change between samples drawn within intervals of 2, 4, 6, or 12 h. Troponin concentrations <3 ng/L (hs-cTnT) and <10 ng/L (cTnI), which represent values less than the limits of detection, were set to 3 ng/L and 10 ng/L.

For calculation of RCVs, hs-cTnT and cTnI values below the limit of detection (3 ng/L and 10 ng/L, respectively) were excluded from analysis. All remaining patients with 2 or more hs-cTnT or cTnI results (57 patients with 188 results for hs-cTnT, 41 patients with 134 results for cTnI) were analyzed. With these data, the mean time between samples was 4.0 h for hs-cTnT

and 4.6 h for cTnI. A change in cardiac troponin concentration that exceeds the RCV (at a 4-h interval for hs-cTnT and 4.6 h for cTnI) can be identified as clinically significant and may suggest acute myocardial necrosis.

RCVs were evaluated with both normal and log-normal approaches as described (13). Using a normal approach, we calculated within-individual biological imprecision (CV_i) from the coefficient of the total imprecision (CV_t) of cardiac troponin at all time points, as follows: $CV_i = \sqrt{CV_t^2 - CV_a^2}$ (Eq. 1). Herein CV_a is analytical variation found to be 7.8% with the Cobas e601 hs-cTnT assay (13) and 10.7% with the Siemens Advia Centaur cTnI (within-run CV as established with a CLSI evaluation protocol at a concentration of 27 ng/L). The limits of the symmetrical RCV were determined as: $RCV = Z \times \sqrt{2} \times \sqrt{CV_a^2 - CV_i^2}$ (Eq. 2), where $z = 1.96$ (z score for 95% confidence). The median normal deviation of the log-normal distribution (σ) was calculated from the median CV_t (as a decimal value), as: $\sigma = \sqrt{\ln(CV_t^2 + 1)}$ (Eq. 3). The asymmetrical limits for the upward (positive) value for the log-normal RCV (RCV^+) and for the downward (negative) value for the log-normal RCV (RCV^-), were calculated as follows: $RCV^+ = e^{(z \times \sqrt{2} \times \sigma) - 1} \times 100$, $RCV^- = e^{(-z \times \sqrt{2} \times \sigma) - 1} \times 100$ (Eq. 4), where $z = 1.96$ (z score for 95% confidence).

The II was calculated with the simplified equation: $II = CV_i / CV_g$ (Eq. 5), where CV_g is the inter-individual CV. We could use the simplified equation because for cTnI and hs-cTnT, CV_a was much smaller than CV_i .

Results

A total of 137 patients completed the original study (15). All patients presented with chest pain and were suspected to have ACS. Of those patients, ACS-NSTEMI was diagnosed in 31, ACS-UAP in 9, and stable AP and chronically increased cTnI in 2 patients. By definition only those patients with cTnI concentrations <60 ng/L and normal ECG findings were included in the present study. Thus, 95 patients were eligible for inclusion; 67 of those 95 patients had ≥ 3 cardiac troponin results for both cardiac troponin assays available and were included in the statistical analyses. Population characteristics and relevant medical history of these patients are depicted in Table 1.

Fig. 1 shows the mean cTnI and cTnT concentrations for each patient. The bars in Fig. 1 depict the range of cardiac troponin for each patient. hs-cTnT was detected in more patients than cTnI, i.e., 50 vs 34 patients ($P < 0.0001$, Fisher exact test). The median cardiac troponin concentrations were 5.5 ng/L for hs-

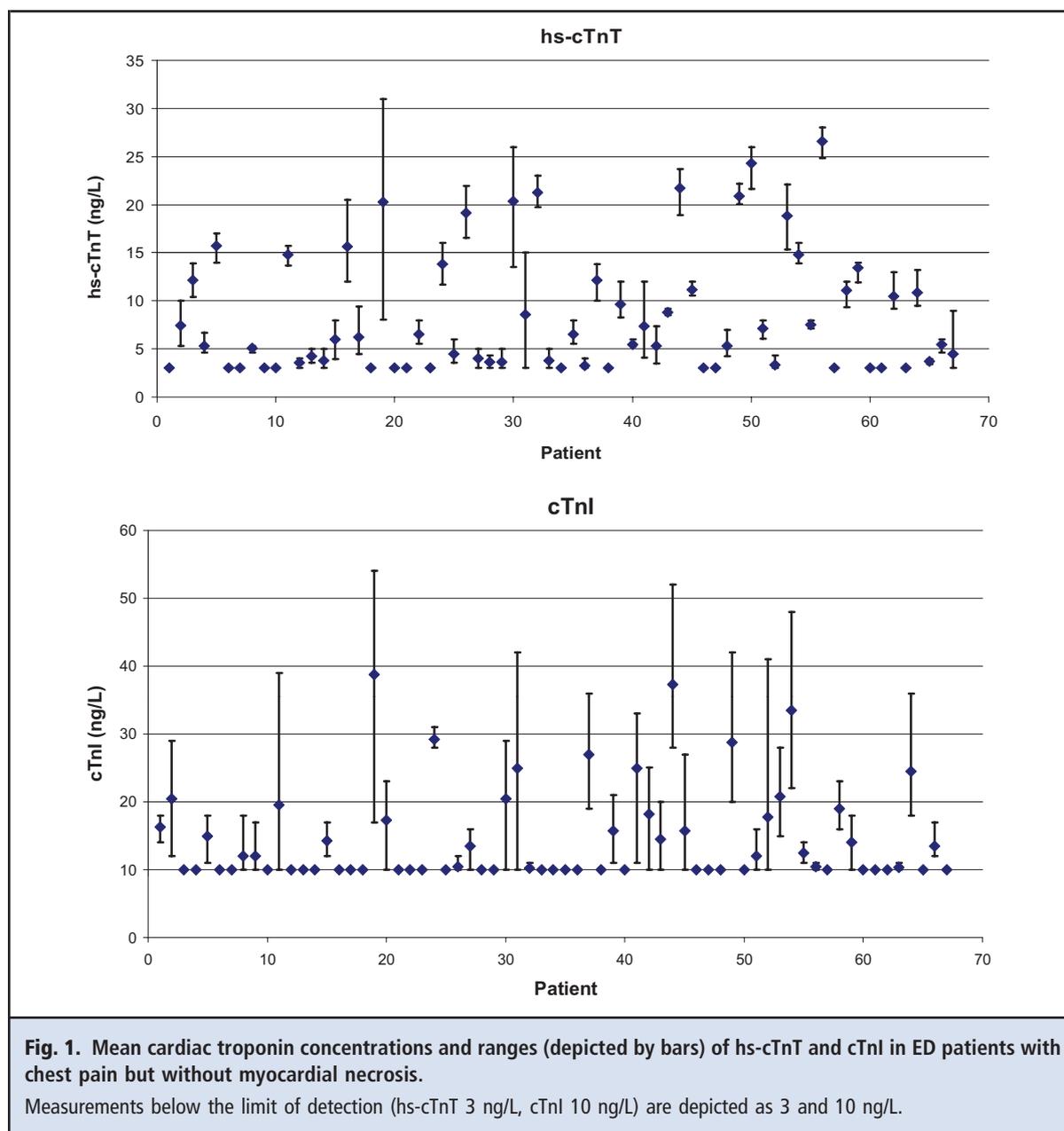
Table 1. Patient characteristics.

No. of patients	67
Age, median (range), years	64 (36–87)
Male sex, %	68
Cardiovascular history, n (%)	46 (69)
Heart failure	0 (0)
MI	22 (33)
AP	6 (9)
Coronary artery disease	9 (13)
Coronary artery bypass graft	5 (7)
Transient ischemic attack/ cerebrovascular accident	4 (6)
No cardiovascular history	21 (31)
Medical history, n (%)	
Diabetes mellitus	10 (15)
Kidney failure	0 (0)
Hypertension	14 (21)
Family history of ACS	6 (9)

cTnT and <10 ng/L for cTnI. In these ED patients without myocardial necrosis, the highest concentrations found were 54 ng/L for cTnI and 31 ng/L for hs-cTnT. The highest concentrations of cTnI and cTnT were measured in the same patient (patient 19, Fig. 1). For both assays the maximum within-patient change was also found in that patient: 23 ng/L for hs-cTnT and 37 ng/L for cTnI.

Table 2 presents the 100th (i.e., the maximum observed change), 99th, 97.5th, and 95th percentiles of change in cardiac troponin concentrations. Percentiles of overall change were calculated from the difference between the highest and the lowest cardiac troponin concentration in each patient. The mean time interval between maximum changes is 6.4 h for hs-cTnT and 5.6 h for cTnI. The percentiles of change within 2, 4, 6, and 12 h were calculated from the changes in cardiac troponin between samples drawn at intervals of 2, 4, 6, and 12. The variation in cTnI was larger than in cTnT. For example, the 95th percentile of overall hs-cTnT change (8.3 ng/L) was about 1.5 times the mean hs-cTnT concentration (5.5 ng/L), whereas the 95th percentile of overall cTnI change (28 ng/L) was at least 2.8 times the mean cTnI concentration (<10 ng/L).

Table 3 presents the CVs (CV_i and CV_t are given as median with the range in parenthesis) and RCVs for cTnI and cTnT as assessed with the hs-cTnT and cTnI assays. For both assays, normal and log-normal RCVs were calculated. RCV values obtained with the log-normal approach are given as the upward and the



downward change value, and the mean between these values (in parentheses). The mean time interval between samples was 4.0 h for hs-cTnT and 4.6 h for cTnI. Because of the higher variation in cTnI concentration, the RCVs for cTnI were larger than those for hs-cTnT. For both cardiac troponins, the means of RCV^+ and RCV^- calculated with the log-normal approach were in good agreement with the RCVs obtained with the normal approach.

Finally, the II was calculated to be 0.31 for cTnI and 0.12 for hs-cTnT. Thus, the II was low for both cardiac troponins, indicating that monitoring serial

changes of cardiac troponin concentration may be helpful in ruling out acute myocardial ischemia in patients presenting with chest pain.

Discussion

This study presents data on variation of cTnI and cTnT in ED patients. On admission the patients were suspected of having ACS, but in the patients whose data were used for the analysis reported here the diagnosis of ACS was finally ruled out. Assuming that patients included in this study had not suffered from a coronary

Table 2. Short-term changes in hs-cTnT and cTnI concentrations.		
	hs-cTnT	cTnI
Overall change		
No. of samples	251	266
100th percentile	23.0 ng/L	37 ng/L
99th percentile	16.0 ng/L	34 ng/L
97.5th percentile	12.2 ng/L	31 ng/L
95th percentile	8.3 ng/L	28 ng/L
Change within 2 h		
No of samples	125	132
100th percentile	23.0 ng/L	24 ng/L
99th percentile	17.0 ng/L	24 ng/L
97.5th percentile	12.3 ng/L	23 ng/L
95th percentile	8.5 ng/L	17 ng/L
Change within 4 h		
No of samples	130	132
100th percentile	6.9 ng/L	17 ng/L
99th percentile	5.9 ng/L	16 ng/L
97.5th percentile	4.4 ng/L	16 ng/L
95th percentile	3.6 ng/L	15 ng/L
Change within 6 h		
No of samples	185	201
100th percentile	16.1 ng/L	37 ng/L
99th percentile	10.2 ng/L	32 ng/L
97.5th percentile	9.6 ng/L	20 ng/L
95th percentile	6.0 ng/L	18 ng/L
Change within 12 h		
No of samples	121	134
100th percentile	9.9 ng/L	31 ng/L
99th percentile	9.2 ng/L	30 ng/L
97.5th percentile	7.6 ng/L	27 ng/L
95th percentile	5.6 ng/L	24 ng/L

event in the week(s) before coming to the ED, the variation in cardiac troponin observed in this study can be attributed to physiological and analytical variation in the absence of acute myocardial necrosis. We found that the II of cardiac troponin in ED patients was low, as has been reported for healthy individuals (11), suggesting that intraindividual variation of cardiac troponin in ED patients is not relevantly higher than in healthy individuals. In contrast to earlier studies in which variation of cardiac troponin in healthy individuals was assessed, in this study we assessed cardiac troponin changes in sick patients without acute myocardial necrosis. Compared to healthy individuals, this group may be the clinically

Table 3. Relative short-term variation of cardiac troponins as found with the hs-cTnT and cTnI assays.		
	hs-cTnT	cTnI
Variable		
No of values	188	134
CVa %	7.8	10.7
CVi %	11 (0–66)	18 (0–49)
CVt %	14 (0–48)	21 (0–50)
RCV %		
Normal	38	57
Log-normal ^a	46, –32 (39)	76, –43 (60)

^a RCV values obtained with the log-normal approach are given as the upward and the downward change value and the mean between these values (in parentheses).

more relevant reference group. This approach is unusual, but not unprecedented, because it has been used to establish RCVs for N-terminal pro-B-type natriuretic peptide (16) and for hs-cTnT in heart-failure patients (17).

Relative and absolute variations in cardiac troponin were calculated for cTnT, as measured by the hsTnT assay (Roche Diagnostics), and cTnI, as measured by the TnI-Ultra assay (Siemens Diagnostics). The 95th percentile of the overall absolute change in hs-cTnT was calculated to be 8.3 ng/L, and the corresponding 95th percentile for cTnI was 28 ng/L. The 95th percentile of cTnT change within 2 h after presentation to the ED was 8.5 ng/L and for cTnI 17 ng/L. Reichlin et al. reported similar changes in their study, i.e., they found optimal discrimination between MI and non-MI within 2 h after presentation at 7 ng/L for cTnT and at 16 ng/L for cTnI (14). The change values reported here and by Reichlin et al. (14) are about one-half of the 99th percentile of the respective cardiac troponin assays (4, 8, 9). We found clinically relevant, relative concentration changes of 40% and 60% for hs-cTnT and cTnI, respectively. These findings are in line with what others have found for hs-cTnT (13) and cTnI (11), although a different cTnI assay was used, and cardiac troponin changes for both studies were measured in healthy volunteers. The RCVs reported by Frankenstein et al. (13) for hs-cTnT are about 10% higher than the values we report here (Table 3). This difference in results may have occurred because Frankenstein et al. used a younger, healthy population in which small absolute changes lead to relatively large changes in terms of percentage. Vasile and coworkers (12) found markedly higher changes than those observed by us and other investigators

(11, 13), most likely for reasons discussed by Frankestein et al. (13).

In the literature 2 approaches are described to discriminate physiological from pathological changes in cardiac troponin; one applies relative changes ((11–14), current study) and the other to absolute changes in cardiac troponin ((14), current study). Reichlin et al. (14) compared the ability of both approaches to identify MI and found that the absolute change is more accurate than the relative change. On theoretical grounds, this finding is quite understandable. RCVs are easily exceeded at low cardiac troponin concentrations that are clinically irrelevant, e.g., an increase of hs-cTnT from 3–5 ng/L (a 66% increase). Using the absolute change in hs-cTnT found in this study, the criterion of an absolute cardiac troponin change is met at an hs-cTnT concentration of 11.3 ng/L, the sum of the lowest reportable cardiac troponin concentration (3 ng/L) plus the 95th percentile of overall change (8.3 ng/L). This concentration is almost as high as the 99th percentile (14 ng/L) used by Reichlin et al. (14) to define MI.

Another aspect that might contribute to the better diagnostic performance of the absolute cardiac troponin change is that the mathematical equation for the RCV calculation (see Eq. 1) uses a single value for the CV_a , which is in fact valid for only 1 cardiac troponin concentration. In practice, however, this single CV_a is applied to the entire normal cardiac troponin range, leading to underestimation of the RCV at low and overestimation of the RCV at high cardiac troponin concentrations. Applying the RCVs found here to the highest cardiac troponin concentrations in the population studied (Fig. 1) translates into an hs-cTnT concentration change of 11.8 ng/L (0.38×31 ng/L) and a cTnI concentration of 31 ng/L (0.57×54 ng/L). Both values lie between the 95th and 97.5th percentiles of the overall absolute cardiac troponin changes found here (Table 2). Thus, the discriminatory capacity of cardiac troponin RCVs and absolute cardiac troponin changes might be comparable in the high cardiac troponin range of the study population. However, owing to the limitations of RCVs in comparison to the absolute cardiac troponin changes discussed above, use of absolute changes may be preferred in clinical practice.

According to the European Society of Cardiology guidelines 2011 (10), clinicians should order cardiac

troponin measurement on arrival of the patient and 3 h later. If the first measurement is above the upper reference limit (99th percentile of a reference population) myocardial necrosis is evident on arrival, and the second measurement serves to differentiate acute (cardiac troponin change above the assay-dependent cutoff) from chronic necrosis (cardiac troponin change below the assay-dependent cutoff). If the second measurement is above the upper reference limit (99th percentile of a reference population) and the cardiac troponin change is above the cutoff (RCV or absolute change), acute myocardial necrosis is diagnosed. If the second measurement is below the upper reference limit (99th percentile of a reference population) but the cardiac troponin change is above the cutoff, the patient should be observed further with additional cardiac troponin measurements 6 and 12 h after arrival. The majority of patients will have cardiac troponin concentrations below the 99th percentile and cardiac troponin changes smaller than the assay-specific cutoffs at 0 and 3 h, so an acute myocardial diagnosis can safely be ruled out. On the basis of our study results we suggest using cardiac troponin changes of 8 ng/L for the hs-TnT and 17 ng/L for the Siemens cTnI assay as cutoff values when cardiac troponins are measured on arrival in the ED and 2 hours later. With the use of this approach, the time between arrival and appropriate management (10), i.e., transfer to the cardiology department in case of acute ischemic damage or work-up of the differential diagnosis, may be shortened and the strain on ED capacity decreased.

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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