Critical difference between serial measurements of CK-MB mass to detect myocardial damage

ROBBERT J. de WINTER,1* RUDOLPH W. KOSTER,1 JAN P. VAN STRAALEN,2 JOZEF P.M.C. GORGELS,2 FRANS J. HOEK,2 and GERARD T. SANDERS2

To assess the critical difference in serial measurements of CK-MBmass and the ability of this critical difference to detect myocardial damage, we studied 110 patients in whom an acute myocardial infarction (AMI) had been ruled out. Blood samples were drawn at 3, 4, 5, 6, 7, 8, 12, 16, 20, and 24 h after onset of symptoms. With a critical difference of 72.6%, an increase of >2.0 μg/L between two CK-MBmass measurements was determined to be significant. Twenty-three of the non-AMI patients had an increase in CK-MBmass >2.0 μg/L, but five of these did not have an abnormal concentration of troponin T (i.e., not >0.1 μg/L). Also among the 110 non-AMI patients, 22 did have an abnormal troponin T value, 18 of whom (82%) also had CK-MBmass increased by >2.0 μg/L. In 20 of the 23 patients with an increase in CK-MBmass >2.0 μg/L, this increase was detected from the values for two samples collected at 5 and 12 h after onset of symptoms. In conclusion, using the critical difference for CK-MBmass defined as an increase >2.0 μg/L detected myocardial damage in patients without AMI.

INDEXING TERMS: variation, source of • creatine kinase • isoenzymes • troponin T

In patients presenting with chest pain at the emergency room, early confirmation or exclusion of the presence of acute myocardial infarction (AMI) is essential for cost-effective triage and timely treatment of these patients [1–4].3 However, electrocardiograms (ECGs) can be misleading in 8% of all AMIs and indeterminate in another 12% [5], and as many as 50% of patients with AMI may initially come to emergency departments with nondiagnostic ECGs [6]. Therefore, the diagnosis of myocardial damage in patients with chest pain may rely on the measurement of biochemical markers.

New assays for early markers such as myoglobin, troponin T, and creatine kinase isoenzyme MB mass (CK-MBmass) are highly sensitive for the diagnosis of AMI [1, 4, 7–9]. In addition, however, these assays can detect small amounts of marker release from the damaged myocardium, and the changes in marker values below the predetermined cutoff value for AMI may also be of diagnostic and prognostic importance [10–13]. This so-called minor myocardial damage is currently defined as an increased concentration of troponin T (>0.1 μg/L) and an increase and decrease of CK-MBmass concentrations that remain below the discriminating limit for AMI [11, 14, 15].

The question is, How large an increase of CK-MBmass can be considered abnormal? To answer this, one must first determine the normal variation between serial measurements in a reference population (of chest pain patients) in samples collected at relevant timepoints. Changes that exceed this normal variation between serial measurements—“significant changes,” or the “critical difference,” or the “reference change”—will depend on the biological, analytical, and preanalytical variation of the biochemical marker under study [16, 17]. To our knowledge, the critical difference for CK-MBmass in patients presenting with chest pain but without AMI has not been studied. Therefore, we undertook to assess the critical difference for serial measurements of CK-MBmass in such patients, using frequent sampling in the first 24 h after the onset of symptoms. We also measured troponin T in the patients and compared the ability of that analyte to detect myocardial damage with the detection based on a change in CK-MBmass greater than this critical difference. The optimal timing for drawing blood samples within these first 24 h to detect myocardial damage was also assessed.

3 Nonstandard abbreviations: AMI, acute myocardial infarction; CK-MB, creatine kinase MB isoenzyme; and CD, critical difference.
Materials and Methods
Subjects and Samples
All consecutive patients with chest pain were seen at the Cardiac Emergency Room, a facility that is equipped with continuous ECG monitoring of patients remaining under observation for a maximum of 24 h. Patients with chest pain suggestive of myocardial ischemia who presented within 5 h after the onset of symptoms were eligible for the study. Exclusion criteria were AMI, severe skeletal muscle damage or trauma, cardiac resuscitation, and inability or refusal to give informed consent. The protocol was approved by the Medical Ethics Committee of our institution.

The final diagnosis of AMI was established at hospital discharge, based on the patient’s clinical history and symptoms, ECG abnormalities, and a typical increase and decrease in the CK-MBmass curve with the peak exceeding 15 µg/L (considering the results from all timepoints). Patients fulfilling these criteria for AMI were excluded from the study. The physicians making the diagnosis were unaware of the troponin T results. Some patients (n = 17) showed CK-MBmass gradually increasing to >7.5 µg/L but not exceeding 15 µg/L; they were included in the calculation of the critical difference (see Discussion).

Blood samples were drawn with an indwelling intra-venous catheter at 3, 4, 5, 6, 7, 8, 12, 16, 20, and 24 h after the onset of symptoms. Blood was collected in 10-mL heparin-coated evacuated collection tubes. The samples were centrifuged twice at 1500 g for 10 min and the cells were discarded; the plasma remaining was aliquoted and stored at −20 °C until further analysis.

Laboratory Analysis
CK-MBmass was measured with the immunochemical method implemented on the ACS:180 analyzer (CIBA Corning, Houten, The Netherlands). The detection limit was 0.65 µg/L and assay linearity extended from 0 to 500 µg/L. Calibrators were supplied by the manufacturer. The upper reference limit was 7.5 µg/L, and the cutoff value for AMI was 15.0 µg/L.

Troponin T was measured with an ELISA (cat. no. 1289055) on an ES300 analyzer (all from Boehringer Mannheim, Mannheim, Germany); calibrators were supplied by the manufacturer. The upper reference limit was 0.1 µg/L, and the linearity range of this determination was 0–15 µg/L.

Critical Difference
For each patient the mean value for CK-MBmass and the total coefficient of variation (CVa) was calculated from the results from all timepoints. The critical difference (CD) was calculated from the 50th, 75th, and 90th percentiles [17] of the CVa values, as follows:

\[
CD_\alpha = z \times \sqrt{2} \times \sqrt{(CV_a^2)}
\]

where \(\alpha\) indicates the 50th, 75th, or 90th percentile, as needed.

Usually, the critical difference is reported for the median CV, with a probability of 95%, giving a z-value of 1.96. The preanalytical variation for the measurement of CK-MB is small [17] and can thus be neglected; therefore, CVa is assumed to consist of only the biological variation (CVb) and the analytical variation (CVa). Once the analytical variation is known, the biological variation can be calculated, given that \(CV_a^2 = CV_b^2 + CV_a^2\).

If the probability of false alarm (the probability that a difference between two measurements is interpreted as indicative of myocardial damage when in fact it is not) is set at values from 0.5 to 0.01, probability curves [16] can be plotted for the CK-MBmass change for CVs for the 50th, 75th, and 90th percentiles.

Analytical Variation
The precision, or total reproducibility, was calculated from the within-run, between-run, and day-to-day variation as described in NCCLS EP5-T2 [18]. We determined these values for four concentration ranges by using EVAL-KIT Software (CKCHL, Tilburg, The Netherlands).

Statistical Analysis
The change in CK-MBmass between 5 and 12 h or between 6 and 16 h after the onset of symptoms was calculated for each patient and reported as means ± SD. Differences were analyzed with the Wilcoxon matched-pairs sign rank test. \(P < 0.05\) was considered statistically significant.

Results
Of the 231 patients eligible for the study, 121 patients were finally diagnosed as having AMI and were excluded from the analysis. Therefore, 110 patients without AMI formed the study group: 70 men and 40 women, median age 64 years (range 38–88). This combination of gender and age is representative for a population presenting with chest pain at the emergency room. The time of presentation of these patients (hours after onset of symptoms, expressed in 6-h periods of 24-h clock time) was 23 between 0000 and 0600, 32 between 0600 and 1200, 34 between 1200 and 1800, and 21 between 1800 and 2400 h.

The CVs and resulting CD values are summarized in Table 1. The 50th percentile CV was 16.5%, the 75th percentile CV 26.2%, and the 90th percentile CV 48.8%. The

| Table 1. CVs and corresponding CDs of CK-MBmass for 110 patients without AMI. |
|-----------------|------------|------------|
|                  | CV, %      | CD, %      |
| Mean ± SD       | 21.4 ± 18.3|            |
| Range           | 2.9–79.8   |            |
| 50th percentile | 16.5       | 45.7       |
| 75th percentile | 26.2       | 72.6       |
| 90th percentile | 48.8       | 135.2      |

* Calculated from the CVs at the corresponding percentiles.
critical differences calculated with these CVs were \( \text{CD}_{p50} = 45.7\% \), \( \text{CD}_{p75} = 72.6\% \), and \( \text{CD}_{p90} = 135.2\% \), respectively.

The total reproducibility (analytical variation) of CK-
MB\textsubscript{mass} determinations at concentrations of 6.4, 9.1, 24.2, and 93.9 \( \mu \text{g}/\text{L} \) was 3.9\%, 2.3\%, 5.4\%, and 4.5\%, respectively. Because the analytical variation of the CK-
MB\textsubscript{mass} assay in the lower concentration range (i.e., \(<15 \mu \text{g}/\text{L}\)) averaged 3.1\%, the total variation in patients without AMI mainly consisted of the biological variation (median, 16.2\%).

For biological variation of patients equal to the values for \( \text{CV}_{p50} \), \( \text{CV}_{p75} \), and \( \text{CV}_{p90} \), separate probability curves (“probabilities of false alarm”) can be drawn (Fig. 1). The probability is shown on the \( y \)-axis, and the relative difference between two measurements is given on the \( x \)-axis. As can be seen, a >75\% change in CK-
MB\textsubscript{mass} is below \( P = 0.01 \) for the \( \text{CV}_{p50} \) curve and is at \( P = 0.05 \) for the \( \text{CV}_{p75} \) curve. With this 75\% difference, and with the mean CK-
MB\textsubscript{mass} value for all of the non-AMI patients determined to be 2.7 ± 1.76 \( \mu \text{g}/\text{L} \), we chose the cutoff value for the change in CK-
MB\textsubscript{mass} as 2.0 \( \mu \text{g}/\text{L} \).

For each patient, we plotted the CV and average CK-
MB\textsubscript{mass} from all timepoints during the 24 h of blood collection and observation (Fig. 2). Among the 110 patients, 23 had an increase in CK-
MB\textsubscript{mass} of >2.0 \( \mu \text{g}/\text{L} \). Of these, 17 patients showed an increase and decline in CK-
MB\textsubscript{mass} concentration, with the peak between 7.5 and 15 \( \mu \text{g}/\text{L} \); the other 6 had an increase in CK-
MB\textsubscript{mass} of >2.0 \( \mu \text{g}/\text{L} \) but the peak was <7.5 \( \mu \text{g}/\text{L} \). Overall, of the 23 patients with CK-
MB\textsubscript{mass} increased by >2.0 \( \mu \text{g}/\text{L} \), 18 also had an abnormal concentration of troponin T (>0.1 \( \mu \text{g}/\text{L} \)) but 5 had normal troponin T values. On the other hand, of the 87 patients with no critical increase in CK-
MB\textsubscript{mass}, only 4 patients had above-normal troponin T; the remaining 83 had normal values for both markers.

Of the 22 patients with an abnormal concentration of troponin T, 18 (82\%) were identified by an increase in CK-
MB\textsubscript{mass} >2.0 \( \mu \text{g}/\text{L} \). One of these 22 patients had moderate renal dysfunction (serum creatinine 159 \( \mu \text{mol}/\text{L} \); all the others—including the 4 patients with an abnormal troponin T but no critical increase in CK-
MB\textsubscript{mass}—had normal renal function.

The increase in CK-
MB\textsubscript{mass} >2.0 \( \mu \text{g}/\text{L} \) was evident at 8 h after the onset of symptoms in 14 patients and at 12 h in another 6 patients. One patient had a sudden, large (14.3 \( \mu \text{g}/\text{L} \)) increase in CK-
MB\textsubscript{mass} at 24 h after the onset of symptoms. For two patients, CK-
MB\textsubscript{mass} concentrations were greatest at admission. In the 87 patients without increases in CK-
MB\textsubscript{mass} >2.0 \( \mu \text{g}/\text{L} \), the variation in the CK-
MB\textsubscript{mass} values was not random. Although one might expect the CK-
MB\textsubscript{mass} concentrations to remain stable over time in these patients, in fact the CK-
MB\textsubscript{mass} values declined between 5 and 12 h (from 2.45 ± 1.52 to 2.39 ± 1.57 \( \mu \text{g}/\text{L} \); \( P = 0.008 \)) and between 6 and 16 h (from 2.55 ± 1.60 to 2.22 ± 1.56 \( \mu \text{g}/\text{L} \); \( P < 0.001 \)).

**Discussion**

New accurate CK-
MB\textsubscript{mass} assays that are highly precise in the lower concentration range make it possible to detect minute quantities of CK-MB released from the ischemic heart. This minor release of CK-MB is expected to result in a small increase in plasma CK-
MB\textsubscript{mass} between 3 and 12 h after the onset of ischemia. By definition, an increase in CK-
MB\textsubscript{mass} larger than that expected from normal variation, “the critical difference,” may signify release from the...
myocardium. Therefore, in the continuous spectrum of changes in serial CK-MB\textsubscript{mass} measurements in chest pain patients, the cutoff value for AMI forms the upper limit and the critical difference forms the lower limit of CK-MB\textsubscript{mass} changes indicative of myocardial damage in patients for whom AMI has been ruled out.

We determined the critical difference for CK-MB\textsubscript{mass} to be 2.0 $\mu$g/L on the basis of our CD\textsubscript{90} of 72.6%, which was very similar to the CD\textsubscript{90} in healthy individuals determined by Costongs et al. [17]. Using this critical difference, we were able to identify 18 of 22 patients (82%) who had an abnormal concentration of serum troponin T.

The mean ± SD CV (21.4% ± 18.3%) we found in the non-AMI patients was in the range of that reported (32.5%) for CK-MB activity in selected patients in a retrospective study [19]. The median variation for CK-MB\textsubscript{mass} we determined, 16.5%, resulting in a critical difference of 45.7%, was just slightly above the value Costongs et al. reported for healthy subjects, 40.1% [17]. The CV\textsubscript{p90} of 48.8% in our study results in a critical difference of 135.2%, which is higher than that reported for healthy subjects (72%) [17]; however, as seen in Fig. 2, most patients with a CV >40% had an average CK-MB\textsubscript{mass} <2 $\mu$g/L. The one patient with a CV of 80% had a CK-MB\textsubscript{mass} of ~4 $\mu$g/L; at 24 h, the CK-MB\textsubscript{mass} suddenly rose to 14.3 $\mu$g/L, suggesting a new episode of ischemia but without symptoms.

In comparison with healthy individuals, higher CVs also could be expected in a group of chest pain patients that included patients with a peak CK-MB\textsubscript{mass} >7.5 but <15 $\mu$g/L. This is in part a problem of the “gold standard” for AMI, because a group of patients for whom AMI is ruled out will include patients with a small increase and peak for CK-MB\textsubscript{mass} below the cutoff value for AMI. Calculating the critical difference in a reference population of chest pain patients in whom an AMI has been ruled out and subsequently excluding patients with a minor increase in CK-MB\textsubscript{mass} leads to circular reasoning, because the definition of “a minor increase” follows from the determination of the critical difference. However, if we assume that the majority of the patients determined not to have AMI do not have myocardial damage, then taking the median or 75th percentile value of the CVs from this group of patients will result in a critical difference that is not much influenced by the higher CVs of the patients with a peak CK-MB\textsubscript{mass} between 7.5 and 15.0 $\mu$g/L (Fig. 2).

Using the probability curves in Fig. 1, one can link the percentage change between two measurements with the probability that the observed change is the result of “normal” or “reference” variation. The level of certainty required for excluding myocardial damage may depend on the clinical situation of the individual patient. Therefore, probability curves such as those in Fig. 1 may aid clinicians in decision-making.

In the 87 patients without an increase in CK-MB >2.0 $\mu$g/L, most showed a slight but statistically significant decline in CK-MB values between 5 and 12 h or between 6 and 16 h after the onset of symptoms. This is not an effect of diurnal variation, because the onset of symptoms was evenly distributed over the day (see Results). Instead, this decline may show a “regression to the mean” effect, because patients with a large increase in CK-MB have been excluded; or perhaps it reflects the effects of hospitalization, in that CK-MB released from skeletal muscle during normal activity before admission (and the corresponding concentrations in plasma) decreases when patients are in bed during evaluation in the hospital; or more-effective clearance of CK-MB may be the cause. In 20 of 23 patients whose CK-MB\textsubscript{mass} increased >2.0 $\mu$g/L, the increase became evident between 5 and 12 h after the onset of symptoms. This implies that, for many patients, CK-MB\textsubscript{mass} determinations in two samples taken 5 and 12 h after the onset of symptoms will help clinicians decide what patient management is called for.

Use of 2.0 $\mu$g/L to indicate a significant increase in CK-MB was suggested previously by Pettersson et al. [20], using a different CK-MB\textsubscript{mass} assay (Tandem ICON QSR CK-MB; Hybritech, San Diego, CA)—although they did not arrive at this value with the concept of the critical difference. In that study, unstable angina patients with a significant increase in CK-MB\textsubscript{mass} >2.0 $\mu$g/L (n = 14) had a higher mortality after 2 and 4 years than did patients without such an increase (n = 20).

The prognostic implications of minor myocardial damage, as detected with CK-MB\textsubscript{mass} or with troponin T and troponin I, have recently been discussed in several other reports [10–13, 21]. In our previous study [13], using a cutoff value of 7.5 $\mu$g/L CK-MB\textsubscript{mass} for minor myocardial damage, we were unable to demonstrate independent prognostic information for CK-MB\textsubscript{mass} in comparison with increased troponin T. Ravkilde et al. [12], using a cutoff of 6.0 $\mu$g/L for CK-MB\textsubscript{mass} with another assay (Novoclone; Dako, Copenhagen, Denmark), found similar independent prognostic information for CK-MB\textsubscript{mass} and troponin T but saw no additional prognostic information once the ECG ST-T changes were considered. However, they did not perform an analysis of CK-MB\textsubscript{mass} changes <6.0 $\mu$g/L. We identified 6 patients in the present study with increased CK-MB\textsubscript{mass} >2.0 $\mu$g/L and peak CK-MB\textsubscript{mass} <7.5 $\mu$g/L whose troponin T had increased to >0.1 $\mu$g/L. If the release of CK-MB and troponin T is the result of the same process in the myocardium, namely, cardiac myocyte necrosis, looking at the CK-MB\textsubscript{mass} release curve “with a magnifying glass” may detect myocardial damage as well as an increased troponin T does in patients who have a clear time of onset of symptoms and a timely admission to the emergency room. Three of the four patients in our study with increased troponin T and “normal” CK-MB values had above-normal troponin T on admission, suggesting an episode of ischemia/necrosis before the one that motivated the patient to seek medical attention. For a long enough interval, the CK-MB\textsubscript{mass} on admission probably had already returned to normal val-
ues. Although impaired renal function may cause falsely positive troponin T results [22], all four of the troponin T-positive/CK-MB-negative patients had normal renal function, as did all but one of the other patients with an above-normal troponin T.

Among the potential limitations of this study is the high prevalence of patients with an AMI (123 of 231) in comparison with the prevalence in other studies. However, our patients presented at a cardiac emergency room and not at a general emergency room or first aid department. Also, because the patients had to present within 5 h after the onset of symptoms, patients with a more gradual or “stuttering” onset of chest pain were excluded. Finally, the study protocol, with all the timed samples, may have biased the attending physicians in preferably including patients whose symptoms had a well-defined onset, who are more likely to have AMI. How this possible bias in patient selection might affect the magnitude of the critical difference is as yet unclear.

We used time of onset of symptoms and not the time of admission as a reference, because time of admission has no biological meaning for studying appearance of markers of ischemia. However, the time of onset of symptoms can be difficult or impossible to establish in some patients, and the time of onset of symptoms may not be the time of onset of necrosis. Indeed, in patients with a less precise time of onset of symptoms or with repeated episodes, a marker that stays increased longer (e.g., troponin T) may better identify patients with myocardial damage in whom CK-MBmass has returned to normal values. Given budgetary restraints and the fact that only one specific cardiac marker is available in most hospitals, it may be worthwhile to extract as much information as possible from serial samples by measuring just one marker. Whether a change in the management of non-AMI patients based of the detection of myocardial damage with CK-MBmass or troponin T will improve the outcome in these patients has not yet been shown in a prospective study.

In conclusion, for patients with chest pain but without AMI, a difference between two CK-MBmass measurements >2.0 μg/L provides evidence of myocardial damage. Using this cutoff value, we identified 23 of 110 non-AMI patients as having myocardial damage. Of the 22 patients with an increased troponin T, 18 (82%) also had an increase in CK-MBmass >2.0 μg/L. In our study, 20 of 23 patients with increased CK-MBmass were detected from the values for two serial samples collected at 5 and 12 h after the onset of symptoms.

We thank the physicians and nurses of the Cardiac Emergency Room for their enthusiasm and support during the study. CIBA Corning and Boehringer Mannheim kindly supplied the analyzers and the necessary reagents. This study was in part supported by a grant from the Dutch Heart Foundation (no. 90–096).

References

