Analytical Characteristics of the AxSYM Cardiac Troponin I and Creatine Kinase MB Assays, Dirk Peetz, Gerd Hafner, and Karl J. Lackner (Institute of Clinical Chemistry and Laboratory Medicine, University Hospital, D-55101 Mainz, Germany; address correspondence to this author at: Institute of Clinical Chemistry and Laboratory Medicine, Johannes-Gutenberg University, Langenbeckstrasse 1, D-55101 Mainz, Germany; fax 49-6131-176627, e-mail peetz@zentralabor.klinik.uni-mainz.de)

The use of biomarkers for the detection of myocardial infarction (MI) has been recently redefined by the American College of Cardiology, the American Heart Association, and the European Society of Cardiology (1, 2). These consensus statements replace earlier recommendations by the National Academy of Clinical Biochemistry and IFCC that proposed the use of two separate decision limits: a higher cutoff for detection of acute MI (AMI) and a lower cutoff for detection of myocardial injury of lower degree, as observed in unstable angina pectoris (3, 4). The new consensus statements redefined MI in that even small areas of necrosis are considered as MI.

Cardiac troponins represent cardiac-specific molecules almost undetectable in the blood of healthy individuals. In addition, current immunoassay techniques for quantification of troponins in blood enable identification of small cardiac injury (1, 2). Cardiac troponins have been designated the preferred biomarkers for the diagnosis of AMI. If cardiac troponins are not available, testing for creatine kinase (CK)-MB, although less cardiospecific, can be used (2). Myocardial injury is assumed if measured concentrations of the respective biomarkers are above the 99th percentile of a healthy reference group, provided that the imprecision (CV) of the assay used is ≤10% at the decision limit (1, 2). Using the new guidelines, we evaluated the AxSYM Troponin-I and CK-MB assays.

Cardiac troponin I (cTnI) and CK-MB were measured by microparticle enzyme immunoassays on a routine AxSYM analyzer (Abbott Laboratories) as described elsewhere (5). The minimum detectable concentration (MDC) of the AxSYM cTnI was evaluated as the mean + 2 SD of zero calibrator in 20 replicates. Total imprecision was assessed by measurement of 10 different serum pools with cTnI concentrations between 0.01 and 1.20 μg/L (corresponding CK-MB, 0.9–8.4 μg/L). cTnI and CK-MB were determined twice daily in independent analyses over a period of 20 working days, using a single lot of reagents and a single calibration curve. Total imprecision was calculated according to NCCLS guideline EP5-A.

Samples from 989 healthy volunteer Caucasian blood donors [461 women (median age, 33.5 years; range, 18–68 years) and 528 men (median age, 41.0 years; range, 19–69 years] obtained from our local blood bank were collected as a reference group. Informed consent was obtained from all participants. The health status of each participant was evaluated based on recorded donor history, a questionnaire, a personal interview by a physician, and routine blood tests. Donors with a suspicious history (e.g., cardiovascular events), pathologic blood analysis, or a body temperature >38 °C were excluded. Serum samples were obtained from tubes containing gel barrier and clot activator (S-Monovette® S; Sarstedt), and plasma was obtained from tubes containing 15 IU of lithium heparinate per 1 mL blood (S-Monovette LH). To ensure complete clotting of blood, serum samples were stored at least 1 h before centrifugation. All samples were centrifuged at 3000g for 10 min, and aliquots were frozen at −20 °C within 4 h. No aliquot was stored longer than 4 weeks before analysis, which is well within the documented stability of cTnI (5). Thawed samples were again centrifuged before analysis.

The MDC was 0.24 μg/L. The 99th percentiles for cTnI results in serum and heparin-plasma samples from the reference group were 0.30 and 0.38 μg/L, respectively [95% confidence intervals (CIs), 0.23–0.42 and 0.26–0.48 μg/L, respectively]. In 4 of 989 samples (0.4%), cTnI results were >0.5 μg/L (upper normal reference value according to package insert). In these cases, another sample aliquot was reanalyzed on the AxSYM and by another troponin assay (TnT on Elecsys 2010; Roche Diagnostics). None of these samples showed increased troponin T (TnT), and the increased cTnI (2.89 μg/L) was confirmed in only 1 sample (2.46 μg/L). A follow-up sample from this individual could not be obtained. In all samples, the CK-MB mass concentration was <5 μg/L. The 99th percentiles for CK-MB mass results in serum and heparin-plasma samples were 7.3 and 8.0 μg/L, respectively (95% CIs, 6.7–9.3 and 6.6–9.5 μg/L, respectively). All individuals with CK-MB concentrations above the calculated 99th percentile (n = 10) showed cTnI concentrations below the respective calculated cutoff, indicating CK-MB increases of noncardiac origin.

Total imprecision (CV) for cTnI was 29% at a concentration of 0.30 μg/L, 20% at 0.45 μg/L, and <10% at ≥0.84 μg/L (Fig. 1). According to the recommendations by Apple and Wu (6), the preliminary cutoff was set at 0.84 μg/L, the cTnI concentration corresponding to a CV of 10%. Importantly, the 95% CIs of the 99th percentile of the reference control group (±0.24–0.47 μg/L) and the CI of the proposed 10% CV cutoff (0.67–1.01 μg/L) did not overlap (Fig. 1). Therefore, using that cutoff value, we could clearly distinguish patients with MI from non-MI patients. The total imprecision (CV) for CK-MB in serum samples was <10% for all samples with concentrations ≥3.1 μg/L.

Troponins are the markers of choice for the biochemical detection of myocardial injury (2). Initially, troponins were used for the detection and/or confirmation of AMI, using relatively high cutoff points, e.g., 0.5 μg/L for the original cTnI (7) and 2.0 μg/L for the AxSYM cTnI assay (8). However, applying significantly lower cutoffs than those used for AMI diagnosis (e.g., 0.1 μg/L cTnI and 1.0 μg/L AxSYM cTnI), subsequent studies revealed that cardiac troponins are powerful prognostic markers for the diagnosis of unstable angina or non-ST-segment elevation
MI (9, 10). The redefinition of MI by international cardiology associations led to a further reduction of the cutoff values. These recommendations suggest a 10% CV at the 99th percentile. It is important to note, however, that this theoretical demand is currently not met by existing commercial troponin assays. The AxSYM Troponin-I assay is one of the most widely used troponin assays worldwide, and valid data on its characteristics in the low measuring range are lacking (11). This study provides reliable data about the performance of the AxSYM assay in relation to the consensus recommendations.

A cutoff point of 0.84 μg/L cTnI is proposed on the basis of the reference group results and on the imprecision data obtained. An imprecision of 29% CV at the 99th percentile of the reference group (0.30 μg/L) does not permit reliable determination of cTnI at this concentration. We consider the lowest cTnI concentration reaching a CV of ≤10% to be the cutoff point (6). In previous studies, CVs of 9.0–10.2% were reported for cTnI concentrations of 1.3–2.9 μg/L (5, 8, 12). These data suggest that the AxSYM cTnI assay shows a plateau of ~10% CV at concentrations between 0.84 and 2.9 μg/L.

Apple et al. (8) reported a 95th percentile for cTnI (n = 437) of 0.5 μg/L, which is higher than observed in our study. However, there is no information about the 95% CI of the 95th percentile cutoff reported by Apple et al. (8). Both cutoffs might overlap with no statistically significant difference between them. Furthermore, because fibrin has been described as a potential interfering factor for cTnI determination (13), we took particular care to thoroughly centrifuge samples before measurements.

The AxSYM CK-MB mass has a CV <10% at the 99th percentile of the healthy control group, thereby meeting the consensus requirements. However, despite the better imprecision profile of CK-MB assays, troponins are recommended as the first-choice markers because of their nearly absolute myocardial tissue specificity (2).

With both assays, cTnI and CK-MB, heparin-plasma samples showed slightly higher values than serum samples at the 99th percentile of the reference group (P <0.05). However, both sample materials can be recommended for cTnI and CK-MB mass determination on the AxSYM analyzer if specific cutoff values are applied.

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References


