Background: Cardiac troponin I (cTnI) is a more specific and sensitive biomarker than creatine kinase MB (CKMB) for detection of myocardial damage. We report the prevalence of positive cTnI and CKMB mass among patients hospitalized with suspected acute coronary syndrome (ACS) and the potential impact of use of different reference cutoffs, particularly those proposed by European Society of Cardiology/American College of Cardiology (ESC/ACC) consensus guidelines, on rates of diagnosis of acute myocardial infarction (AMI).

Methods: We analyzed 1719 consecutive patients with suspected ACS admitted to an urban acute care hospital over a 6-month period. Patients (≥18 years of age) had at least two separate sets of plasma biomarkers (cTnI and CKMB) measured more than 12–24 h after admission to determine the potential rates of AMI based on different biomarker cutoff concentrations.

Results: The prevalence of cTnI-positive cases ranged from 10.6%, based on a cutoff of twice the ROC curve (cTnI < 1.2 μg/L), to 25.0%, using the ESC/ACC-recommended 99th percentile cutoff (cTnI < 0.1 μg/L). The prevalence of CKMB-positive cases ranged from 10.4%, with the cutoff of twice the ROC curve (CKMB ≤ 10.0 μg/L) to 21.7%, with the 99th percentile cutoff (CKMB < 3.9 μg/L). Use of the 10% CV cutoff (cTnI ≤ 0.3 μg/L and CKMB < 3.9 μg/L) instead of the ROC cutoff produced a 26% increase in all cTnI-positive cases. Use of the 99th percentile reference cutoff instead of the ROC curve-derived cutoff produced an 85% increase in all cTnI-positive cases. A substantial proportion of the increase in total cTnI-positive cases was derived from cTnI-positive/CKMB-negative cases: 71 (4.1%), 73 (4.2%), 98 (5.7%), and 209 (12.2%) of cTnI-positive cases were CKMB-negative, as determined by the twice the ROC, ROC, 10% CV, and 99th percentile reference cutoffs, respectively. At the 99th percentile cutoffs, 8.8% of cases were CKMB-positive/cTnI-negative.

Conclusions: Use of lower reference cutoffs for plasma biomarkers, as recommended by ESC/ACC guidelines, markedly increases the rates of cTnI-positive cases overall. A substantial proportion of the increase in total cTnI-positive cases was derived from the creation of additional cTnI-positive/CKMB-negative cases. CKMB-positive/cTnI-negative cases are likely false positive for myocardial injury.
cardiac troponin standard in clinical practice, some reports have shown that the increased sensitivity and specificity of cTnI and cTnT may yield substantially higher rates of AMI diagnoses (15–19). This is primarily attributable to the creation of additional AMI diagnoses from cases that test positive for cTnI or cTnT but negative for CKMB. In this study we evaluate the potential effects of use of various cTnI cutoff concentrations on the rates of AMI diagnosis in a large patient cohort with suspected ACS.

**Materials and Methods**

Hennepin County Medical Center, a 400-bed urban teaching hospital that provides acute care for the city of Minneapolis, MN, was the site of enrollment of patients with symptoms suggestive of ACS, after Institutional Review Board approval. From September 16, 2001, through March 28, 2002, we enrolled 1719 unselected consecutive patients who were being prospectively evaluated for AMI. Enrollment included patients presenting with chest pain as well as other clinical features considered indicative of ACS. Final discharge clinical diagnoses and electrocardiograms were not obtained. Waste blood specimens collected from all 1719 patients were used for analysis of cTnI and CKMB. As part of an Institutional Review Board-approved substudy to address clinical outcomes and risk assessment, chart reviews allowed preliminary observations to be made about the presence or absence of skeletal muscle injury in the cTnI-negative/CKMB-positive group.

cTnI and CKMB mass were measured (Dade-Behring Dimension RxL) in heparinized plasma specimens obtained from each patient at admission and serially at 4, 8, 12, and 24 h after admission (8). Maximum concentrations for each assay obtained over the 24-h period were used for analysis. A minimum of two serial samplings, with at least one obtained at 12 h after presentation, was required. For cTnI the manufacturer’s stated detection limit is 0.04 μg/L. The ROC curve medical decision cutoff value for AMI was ≤0.6 μg/L (20). The 99th percentile of a reference population was <0.1 μg/L (21). The total imprecision cutoff representing a 10% CV was ≤0.3 μg/L (22). In addition, total imprecision was 8.5% at 0.6 μg/L and 13% at 0.15 μg/L. For CKMB, the manufacturer’s stated detection limit is 0.5 μg/L, with a (total imprecision) 10% CV at <3.9 μg/L as determined in our laboratory. The ROC curve medical decision cutoff value for AMI was ≤5.0 μg/L (23), and the 99th percentile of a reference population was <3.9 μg/L (21). Because the lowest concentration for CKMB that demonstrates a CV ≤10% was below the 99th percentile, the same cutoff concentration (<3.9 μg/L) was used for both limits.

Differences in mean maximum cTnI and CKMB concentrations between cTnI-positive/CKMB-positive and cTnI-positive/CKMB-negative cases were compared by Student t-test (two-sided). Throughout the study, a P value <0.05 was considered significant. All statistical
analyses were performed with SAS for Windows software, Ver. 8.02.

**Results**

The mean (SD) age of the 1719 suspected ACS patients for these admissions was 58.4 (14.7) years (median, 57.3 years; range, 18–98 years). Of these patients, 57.1% (n = 983) were male. Whites accounted for the majority of these admissions, comprising 52.1% of admissions. Blacks, Native Americans, Hispanics, and Asians comprised 30.3%, 7.0%, 3.3%, and 2.3% of admissions, respectively. Other ethnicities accounted for 5.0% of admissions.

Of the 1719 suspected ACS admissions, 183 (10.6%), 232 (13.5%), 293 (17.0%), and 430 (25.0%) cases were cTnI-positive, as determined by cutoffs based on twice the ROC (cTnI ≤0.2 µg/L), the ROC (cTnI ≤0.6 µg/L), 10% CV (cTnI ≤0.3 µg/L), and the 99th percentile (cTnI ≤0.1 µg/L), respectively (Table 1). Similarly, 179 (10.4%), 298 (17.3%), 373 (21.7%), and 373 (21.7%) were CKMB-positive, as determined by the twice the ROC (CKMB =10.0 µg/L), ROC (CKMB =5.0 µg/L), 10% CV (CKMB <3.9 µg/L), and 99th percentile (CKMB <3.9 µg/L) cutoffs, respectively. Depending on which reference cutoff was used, 6.5–12.9% of cases were cTnI-positive/CKMB-positive, whereas 4.1–12.2% of cases were cTnI-positive/CKMB-negative. Therefore, compared with the ROC cutoff, the 10% CV cutoff yielded 26% more cTnI-positive cases overall and 34% more cTnI-positive/CKMB-negative cases. Compared with the ROC cutoff, the 99th percentile cutoff produced 85% more cTnI-positive cases overall and 186% more cTnI-positive/CKMB-negative cases. Furthermore, in the cTnI-positive/CKMB-negative cases and in the overall cTnI-positive cases, we observed 113% and 47% increases in cases, respectively, between the 10% CV and the 99th percentile cutoffs.

As reference cutoff concentrations decreased, no consistent pattern was observed in cTnI-negative/CKMB-positive cases, which comprised 3.9%, 8.1%, 10.4%, and 8.8% of all admissions, as determined by the twice the ROC, ROC, 10% CV, and 99th percentile cutoffs, respectively. Table 1 also shows the total number of positive cases when a cutoff of twice the ROC was used: 179 cases (10.4%) were detected for CKMB and 183 cases (10.6%) for cTnI, with an approximately equal number of cTnI-positive/CKMB-negative cases. Compared with the ROC cutoff, 99th percentile cutoffs were set at 1.2 µg/L, 0.6 µg/L, 0.1 µg/L, and 0.05 µg/L.

As reference cutoff concentrations decreased, no consistent pattern was observed in cTnI-negative/CKMB-positive cases, which comprised 3.9%, 8.1%, 10.4%, and 8.8% of all admissions, as determined by the ROC, 10% CV, and 99th percentile cutoffs, respectively. Table 1 also shows the total number of positive cases when a cutoff of twice the ROC was used: 179 cases (10.4%) were detected for CKMB and 183 cases (10.6%) for cTnI, with an approximately equal number of cTnI-positive/CKMB-negative cases (n = 71; 4.1%) and cTnI-negative/CKMB-positive cases (n = 67; 3.9%) cases.

Among cTnI-positive cases, the mean maximum cTnI concentration for each hospital admission was 12 to 25 times higher (P <0.0001) in CKMB-positive than CKMB-negative cases (Table 2). Distribution analysis of maximum cTnI values by box plots (Fig. 1) demonstrated that median values for maximum cTnI were substantially higher among CKMB-positive than CKMB-negative cases: 6.2 vs 1.3 µg/L, 5.7 vs 0.8 µg/L, and 2.9 vs 0.3 µg/L, as determined by the ROC, 10% CV, and 99th percentile cutoffs, respectively. Maximum cTnI concentrations for cTnI-positive/CKMB-positive cases were skewed toward...
much higher concentrations compared with those for cTnI-positive/CKMB-negative cases.

Discussion
This study shows that of the 1719 hospital admissions to an urban hospital for suspected ACS over a 6-month period, there was an ~17% prevalence [based on the 10% CV cutoff used in clinical practice by the hospital (14)] of cTnI-positive cases. However, depending on which reference cutoff is chosen, this prevalence can be as low as 13.5% (ROC cutoff) to as high as 25% (99th percentile cutoff; Table 1). Although the majority of these cTnI-positive cases were also CKMB-positive, the prevalence of cTnI-positive cases that were CKMB-negative accounted for 4.2–12.2% of all ACS admissions, which is nearly the proportion of cTnI-positive/CKMB-positive cases (Table 1). It has been proposed that among ACS patients, troponin-positive/CKMB-negative cases probably represent non-Q-wave or non-ST-elevation myocardial infarction diagnosed by cTnI or cTnT that would have been diagnosed as unstable angina by CKMB (3). Thus, adoption of lower biomarker reference limits, such as the 99th percentile cutoff endorsed by the ESC/ACC guideline (1, 2, 4), produces additional cTnI-positive/CKMB-negative cases that probably represent mild cases of AMI. Therefore, lower biomarker reference cutoffs will contribute to increased rates of AMI diagnoses (17–19). Any increases in cTnI or CKMB above the different cutoff concentrations were considered indicative of a MI. We acknowledge that in clinical practice, careful review of the clinical data would be prospectively included in the context of minor biomarker increases before diagnoses were determined. Determination of clinical outcomes was not part of this study design, but the clinical significance of lowering the MI cutoff to the 99th percentile has been supported by studies that have demonstrated added risk stratification for adverse outcomes at cardiac troponin concentrations above the 99th percentile but below both the 10% CV and the ROC curve cutoffs (12, 13, 24). In the current study, this would have included an additional 137 patients (47% increase) between the 99th percentile and 10% CV cutoffs and an additional 61 patients (26% increase) between the 10% CV and ROC cutoffs, with an overall increase of 85% between the ROC and 99th percentile cutoffs.

On the basis of the 99th percentile cutoffs, the prevalence of CKMB-positive cases among ACS patients in our hospital was 21.7%. However, only 40% of these were cTnI-positive. These cases should be considered representative of AMI. The specificity of cTnI for diagnosis of AMI has been established as superior to that of CKMB.
As such, 60% of the cases that were CKMB-positive in our study may be considered false positives for AMI. Preliminary analysis of these CKMB-positive/cTnI-negative cases suggests that they can be explained by the presence of trauma, rhabdomyolysis, generalized seizures, myopathies, and other mechanisms of injury to skeletal muscle that cause increases in CKMB in the absence of myocardial damage (4–6, 26). Supporting our categorization of these cases as false positive for myocardial injury, there is no literature to our knowledge that has demonstrated a MI case that shows increased CKMB with a normal cardiac troponin.

When only a single, maximum CKMB concentration is used as a diagnostic biomarker for MI detection in the clinical setting, for epidemiology trending, or for clinical trial enrollment or endpoint criteria, caution is suggested regarding the potential of false-positive CKMB results as being indicative of and interpreted as myocardial injury. Although not part of the current study design, trending rising and falling CKMB patterns would likely assist in distinguishing true-positive CKMB increases from false-positive CKMB increases. Interestingly, when twice the ROC cutoff concentrations for both CKMB and cTnI were used, as are commonly used in clinical or epidemiology trials, an approximately equal number of cTnI-positive/CKMB-positive cases were identified overall, with no difference found when the alternative biomarker was negative (Table 1). This observation again demonstrates how event rates can be confounded based on biomarker cutoffs that are used for study enrollment or endpoint criteria.

ESC/ACC consensus guidelines (1, 2), in agreement with the IFCC (27), have specifically proposed that the upper reference limit of normal for cardiac troponin measurements should be the 99th percentile of a reference control group. Furthermore, it has been agreed on that all cardiac troponin assays should meet a total imprecision of 10% CV at the 99th percentile. Therefore, in the clinical setting of myocardial ischemia, any increase of cTnI or cTnT during the first 24 h after the index event is considered an appropriate criterion for the diagnosis of AMI (1–4, 27). In the current study, serial sampling over 12–24 h after admission was used to determine the maximum cTnI or CKMB concentrations and, thus, to determine the rate of MI. Therefore, whether the patient was triaged as an inpatient or outpatient would not impact the overall findings of our study. Current commercial assays, however, cannot attain the ≤10% CV imposed by standard requirements for precision (14). This shortcoming probably contributes to the lack of uniformity among hospitals and emergency departments in their adoption of reference cutoffs for the various commercially available cTnI and cTnT assays used to diagnose AMI. It has been proposed that until ≤10% CV can be attained at the 99th percentile reference limit, the lowest concentration that produces a CV of 10% should be adopted as the diagnostic cutoff (14, 25). Our study has demonstrated that adopting a 99th percentile reference cutoff for cTnI, in compliance with the current ESC/ACC guideline, may yield substantial increases in AMI diagnoses. Replacing the ROC with 99th percentile reference cutoff would increase cTnI-positive cases by 85%. A more modest but substantial increase of 26% in cTnI-positive cases would result if the ROC cutoff is replaced with the 10% CV cutoff.

Our study has shown that depending on which reference cutoff is used, substantially different rates of AMI may be attained, largely as a result of the inclusion of additional cTnI-positive cases that are CKMB-negative. The 99th percentile cutoff, compared with the ROC cutoff, produced an 85% increase in cTnI-positive cases, with 69% of this increase derived from cases that are cTnI-positive/CKMB-negative. These cTnI-positive/CKMB-negative cases increased by 186% when the reference limit was decreased from the ROC to the 99th percentile cutoff. These data are consistent with results of previous studies, which have shown that cardiac troponin assays yield higher rates of AMI compared with CKMB because of the classification of additional troponin-positive/CKMB-negative cases (15, 16). Our data underscore the importance of choosing appropriate reference cutoffs for AMI diagnosis because they can potentially have profound effects on the AMI rates that result. The cTnI assay used in the current study (Dade-Behring Dimension) represents a second-generation assay with good low-end analytical precision characteristics (23). However, because different cardiac troponin assays display variable imprecision at the low concentration limit, the findings of our study should not be translated to other assays without confirmation (14). However, we anticipate similar trends of increasing numbers of increased MI rates based on increased cardiac troponin findings for all cardiac troponin assays compared with CKMB (28).

In this study we do not present actual clinically diagnosed AMIs, but biomarker diagnoses, with special attention given to the prevalence of positive cTnI among suspected ACS admissions. This may perhaps be the main limitation of our study because cTnI can become positive in any pathologic condition that causes myocardial damage, such as myocarditis and severe pulmonary thromboembolism, and not be truly representative of AMI (4–6, 26). Nevertheless, because our aim was to illustrate how use of different reference cutoffs can produce markedly varied rates of AMI, our study using a large patient cohort presents rates of positive cTnI among suspected ACS patients to serve as estimates of biomarker-positive AMI. It should be re-emphasized, however, that increases in cardiac troponins specifically detect the presence of myocardial injury and that these increases, in the clinical setting of cardiac ischemia, define AMI (1–4, 25).

In summary, we have shown that use of lower reference cutoffs for plasma cardiac troponin, as recommended by ESC/ACC and American Heart Association/ACC guidelines, markedly increases the rates of cTnI-positive hospitalizations for suspected ACS. A substantial proportion of the increase in these cTnI-positive cases was derived from
an increased number of cTnI-positive/CKMB-negative cases. These findings serve to suggest that implementing these guidelines for adopting lower biomarker reference cutoffs may substantially increase rates of diagnosis of AMI. Furthermore, we demonstrate a high false-positive rate for AMI among CKMB-positive cases: approximately one-half of them were cTnI-negative. Thus we recommend caution when only CKMB is used as a diagnostic criterion. These findings could have an overall impact regarding patient management, therapeutic decisions in the assessment of risk in ACS patients, in medical reimbursement, in clinical trial outcomes, and in epidemiologic surveys for rates of detection of AMI.

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References


