Assessing the Requirement for the 6-Hour Interval between Specimens in the American Heart Association Classification of Myocardial Infarction in Epidemiology and Clinical Research Studies

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Background: The American Heart Association (AHA) case definition for acute myocardial infarction (AMI) requires an "adequate set" of biomarkers: 2 measurements of the same marker at least 6 h apart. A sensitive troponin assay might detect significant changes in concentration earlier. We determined AMI prevalence, using protocols with shorter intervals between measurements, with and without incorporating the time from onset of symptoms.

Methods: The AHA case definition was used to retrospectively assign a diagnosis in 258 patients presenting to the emergency department with symptoms of cardiac ischemia. AMI was diagnosed if either specimen in an adequate set had a cardiac troponin I (cTnI) above the 99th percentile (AccuTnI® >0.04 μg/L; Beckman Coulter) with a ≥20% change in concentration between specimens. We assessed positivity for AMI after progressively decreasing the time interval between specimens in specimen sets. In addition, for each patient, 2 additional specimen pairs were selected: pairs collected at least 1 h apart with 1 specimen being either ≥3 h after onset or ≥6 h after onset.

Results: When we used the AHA definition, the AMI prevalence was 35.7%. Prevalence was not significantly diminished when the interval between specimens was ≥5, ≥4, or ≥3 h (36.4%, 34.5%, and 33.7%, respectively) compared with the AHA ≥6 h interval. When the time from onset of symptoms was included in the specimen selection algorithm, a 1-h interval was sufficient provided that at least one specimen was collected ≥6 h after onset (prevalence, 34.1%; *P* = 0.48 vs AHA definition).

Conclusion: A sensitive cTnI assay in specimen sets with time intervals ≥3 h, or having one specimen ≥6 h after onset, gave an AMI prevalence equivalent to the AHA definition.

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In late 2003, a Scientific Statement was published by the American Heart Association (AHA)[7] to standardize and update case definitions of acute coronary heart disease (CHD) *(1)*. Consistent application of the definition for CHD is a key determinant of the incidence and prevalence, not only within a population, but also between populations that differ in location or date. The AHA statement provided definitions of ischemic CHD based on the European Society of Cardiology/American College of Cardiology (ESC/ACC) redefinition of acute myocardial infarction (AMI) *(2, 3)*, and specified how to use cardiac biomarkers, cardiac symptoms and signs, and electrocar-

diographic (ECG) findings to classify an individual as having a definite AMI. An important change was that a specific biomarker, such as troponin, could classify a patient as having an AMI even in the absence of abnormal ECG findings if there was strong evidence of ischemia *(1)*. For this to occur, a "diagnostic set" of biomarkers had to be in evidence. This entailed having 2 or more measurements of the same biomarker in specimens obtained at least 6 h apart, with at least 1 measurement exceeding the 99th percentile of a healthy population, and with a rising or falling pattern *(1–3)*. The authors of the Scientific Statement called for comparison studies to test, evaluate, and validate these definitions.

In the acute-care setting, clinicians may not be particularly sensitive to the requirement for an interval between samples of at least 6 h and may wish to act earlier in the interest of reaching a timely diagnosis or a decision about immediate care. Such a strategy is supported by the data of Eggers et al. *(4)*, who have shown that many patients have increased biomarker concentrations long before 6 h. However, the effect of using such a strategy more globally in an unselected cohort is unclear. To examine the extent to which shorter interval protocols might be possible without inducing inaccuracies in the diagnostic paradigm, we retrospectively tested an existing sample bank at shorter time intervals and compared the results with the AHA-defined prevalence. In addition, we examined the potential benefit of including the time of onset of symptoms as a determinant for specimen scheduling.

Materials and Methods

PATIENT SELECTION

In 1996, after receipt of institutional ethics approval, 455 patients representing 500 separate patient presentations to the Emergency Department (ED) were enrolled in a Cardiac Markers Study at the Oshawa General Hospital (now Lakeridge Health Corporation, Oshawa site). The only inclusion criterion was the assessment by the triage staff during recruitment in the ED that the patient was having symptoms suggestive of cardiac ischemia. There were no clinical exclusion criteria. After consent was provided for the collection of study specimens, information about the nature and time of onset of symptoms was solicited. Specimen collections were scheduled for the duration of the patient's stay, as follows: heparinized plasma specimens were collected at presentation, and hourly thereafter until 6 h after the time of onset, and thereafter at 9, 12, 24, and 48 h after onset. A time of onset was obtained in ~92% of presentations; in those patients who could not provide a time of onset, the time of presentation was used. Specimen collections were scheduled to the nearest 10 min and were recorded after rounding the actual time to the nearest hour of elapsed time from onset.

The presentation and 12, 24, and 48 h specimens (if collected) were used for the clinical decisions made in 1996, in accordance with the standards of care at that time.

Treatment decisions were based in part on the measurement of total creatine kinase activity (Bayer Axon) and creatine kinase-MB mass concentration (Abbott IMx) at presentation and at 12-h intervals until discharge or until 72 h after presentation unless additional measurements were ordered for clinical purposes. Patient care was delivered without bias from the study, and patients were discharged, held in the ED, or admitted without regard for the scheduled collections of study specimens. The specimens obtained thus reflected patient availability under the unbiased clinical judgement at the time. Study specimens were collected according to the scheduled intervals until the patient was discharged, declined further participation, or was removed from the study by those responsible for his or her care. The time delay between onset of symptoms and presentation was also a factor affecting the availability of specimens because the scheduled collections were timed from the onset of symptoms and not presentation. All specimens were stored frozen (below −20 °C) pending study analyses. The patient cohort for this study is also described in a report on the prevalence of AMI in this population *(5)*, using both the original 1996 markers and cutoffs and current biomarkers and cutoffs.

In 2003, Research Ethics Board approval was obtained to reanalyze the banked specimens for cardiac troponin I (cTnI) with the AccuTnI® assay (product no. 33340) from Beckman Coulter. Specimen stability has been well documented with the AccuTnI troponin assay after multiple freeze–thaw cycles *(6, 7)*. For the purposes of the present study, additional inclusion criteria were applied to the original cohort of 500 patient presentations to comply with the AHA case definition of AMI. After exclusion of 11 presentations that were >24 h after the onset of symptoms and the presentations without a specimen interval of at least 6 h as required by the AHA Statement *(1)*, 258 patients were available for the study [mean (SD) age, 65.8 (15.0) years; fraction admitted, 94%]. The prevalence of AMI, according to the AHA case definition, in this subset of the population was 35.7% (99th percentile cTnI cutoff, with 20% change criterion), which included all 26 patients with ST-elevation myocardial infarction (STEMI). The details of that prevalence analysis have been reported elsewhere *(5)*. Each patient in the present study had, at a minimum, specimens collected at presentation and 6 h thereafter, thus complying with the AHA definition of an adequate specimen set. These AHA-compliant specimen sets were used for reference, and comparisons were made with the positivity of specimen sets derived from the application of alternative definitions of specimen intervals.

The reliability of the time of onset data was assessed by independent chart reviews of all instances (46 of 497) of presentations with any of the following: missing times of onset, missing times of ECGs, unlikely times of onset (e.g., exactly midnight), or onset to presentation intervals <10 min. There were 20 patients for whom the time of onset

was neither recorded in the study data nor available from the chart review; however, each of these patients had specimens collected at recorded intervals from onset. Of these, 19 had their first specimen listed as 1 h after onset, and 1 had the first specimen recorded as 0 h after onset. We could not find any clinical information to confirm or refute these collection times in these 20 patients; we therefore used the listed 1996 times from onset in our analyses.

CUTOFF CONCENTRATION FOR cTnI

The AHA case definition uses a cutoff concentration for troponin set at the 99th percentile of a reference population or the concentration at which the assay achieves a CV of 10% if that exceeds the 99th percentile (1, 8). For the AccuTnI assay, the 99th percentile concentration reportedly is 0.04 μg/L, and the assay achieves a 10% CV at a concentration of 0.06 μg/L (9). Our cTnI measurements were performed on the Access® Immunoassay System from Beckman Coulter, and we determined that the detection limit of our assay was 0.009 μg/L (10). From this, and from a precision profile of 6 commercial quality-control samples, we confirmed that our analyzer achieved a 10% CV at, or very close to, the reported concentration of 0.06 μg/L (9).

CRITERIA FOR AMI CLASSIFICATION

The AHA case definition (1) was used to identify patients with AMI. The AHA Scientific Statement defines an adequate set of biomarkers as at least 2 measurements of the same marker taken at least 6 h apart; accordingly, we selected the presentation specimen and the first specimen collected at least 6 h thereafter. For a diagnostic set of biomarkers, one or more of the specimens in an adequate set must have an increased biomarker concentration, and the concentrations must display a rising or falling pattern in the setting of clinical cardiac ischemia. In the present study, an increased cTnI concentration was defined as >0.04 μg/L, the 99th percentile of a reference population (9). A rising or falling pattern was defined as a 20% proportional change relative to the presentation concentration (5).

SELECTION OF SPECIMEN SETS WITH DIMINISHED TIME INTERVALS

We examined the requirement for a 6-h interval between specimens in an adequate set by successively reducing the defined minimum interval between specimens from 6 h (the AHA standard) down to 1 h. Each new trial definition of a specimen set was applied separately to the specimen bank, selecting for each of the 258 patients the presentation specimen and first subsequent specimen that met or exceeded the amended minimum interval definition. Positive results for the amended minimum interval sets were defined with the same cutoff and change criteria used for the AHA-compliant specimen sets, and the results were compared with those from the AHA sets from the same

258 patients. (A subset of specimens was also selected with intervals corresponding to, but not greater than, the amended minimum intervals. The positivity in these specimen sets was not significantly different from the minimum interval sets; therefore, only the data for the minimum interval sets are presented.)

INCORPORATING TIME OF ONSET IN THE SELECTION OF SPECIMEN SETS

The utility of a specimen set with a minimum 1-h interval was further examined, this time with the additional requirement for a minimum time from symptom onset for one of the specimens in the set. Each of the 258 patients was assessed by use of 2 candidate specimen sets constructed as follows: the presentation specimen and the first subsequent specimen at least 1 h later that was also (a) ≥3 h after the reported onset of symptoms or (b) ≥6 h after onset. Positive results were defined as before, and the numbers of positive sets were again compared with the AHA protocol in the same population of 258 patients. The principal difference in this assessment was that the AHA adequate set used a 6-h interval after presentation, whereas in the study protocol the interval was timed from the onset of symptoms. For each of these sets, the required time to diagnosis (TTD) was calculated as the time from the presentation specimen to the time of the second specimen in the set, without incorporating an assay turn-around time. The TTDs for the candidate time-from-onset specimen sets were subtracted from the identically calculated interval in the AHA-adequate sets to estimate the reduction in TTD afforded by the time-from-onset protocols.

STATISICAL ANALYSIS

For each prevalence calculation, the 95% confidence interval (95% CI) was derived by use of a modified Wald confidence interval of a proportion (11). Concordance among the various specimen sets (e.g., between the AHA and a diminished minimum interval specimen set) was determined by the McNemar test (12) with P values <0.05 considered significant. No adjustment for multiple comparisons was made.

Results

The number of specimen sets at each interval (the "actual interval") when the specified "minimum interval" between specimens in a set was progressively decreased from 6 h (the AHA definition) to 1 h is shown in Table 1. By design, the study included all 258 patients in each of the 6 minimum-interval definitions; however, not all patients had specimens collected at each hour. Therefore, as the specified minimum interval between specimens was decreased, in some instances the same specimen sets were reselected, reflecting the fact that the intervals are defined by their minimum time; e.g., when the interval was defined as at least 6 h, the average time interval was 8.4 h in our population (see Table 1).

Table 1. Distributions of the actual intervals between specimen sets that resulted from progressively decreasing the required interval between specimens from the ≥6 h requirement of the AHA case definition in the 258 patient presentations.

Actual interval, h	Specified minimum interval between specimens					
	1 h	2 h	3 h	4 h	5 h	6 h[a]
1	181					
2	19	164				
3	33	49	174			
4	3	18	28	123		
5	0	1	19	26	75	
6	0	1	6	57	59	78
7–9	2	5	10	19	88	140
10–12	8	8	9	11	13	16
13–24	11	11	11	21	22	23
>24	1	1	1	1	1	1
Median, h	1	2	3	5	6	7
Mean, h	2.8	3.8	4.8	6.5	7.6	8.4
95% CI, h	2.2–3.5	3.2–4.4	4.2–5.4	5.9–7.1	7.1–8.1	7.9–8.9

[a] Actual interval distribution when the AHA adequate set definition was applied.

Comparisons between AMI prevalence with the AHA-specified interval (minimum of 6 h) and the prevalences obtained with the diminished intervals are shown in Fig. 1. Concordance between the AHA case definition and that of the diminished intervals was not significantly different until the interval between specimens was decreased to 2 h (P values are provided in Fig. 1). The fraction of the specimen sets that was positive with a minimum interval of 3 h was 33.7% (95% CI, 28.2%–39.7%), compared with 34.5% (28.9%–40.5%) at 4 h and 36.4% (30.8%–42.5%) at 5 h. None of these was significantly different from the 35.7% (30.1%–41.7%) prevalence of AMI when the specimens were at least 6 h apart and met the AHA definition of an adequate set ($P > 0.05$). However, with a 2-h interval, the fraction positive decreased to 31.4% (26.0%–37.3%;

$P = 0.037$), and at 1 h it was 26.7% (21.7%–32.5%; $P < 0.001$).

The results of the 2 specimen collection protocols that incorporated the time from onset as a determinant for specimen collection are shown in Fig. 2. Both of these protocols used a minimum of 1 h between specimens in a set and incorporated the requirement for one specimen to be at least 3 h or at least 6 h from the onset of symptoms. Compared with the 92 patients (35.7% of 258) who had AMI according to the AHA case definition, the minimum 6 h from onset protocol identified 88 patients (34.1%; 95% CI, 28.6%–40.1%; $P = 0.48$), representing 62 patients with non–ST-elevation myocardial infarction (NSTEMI) and all
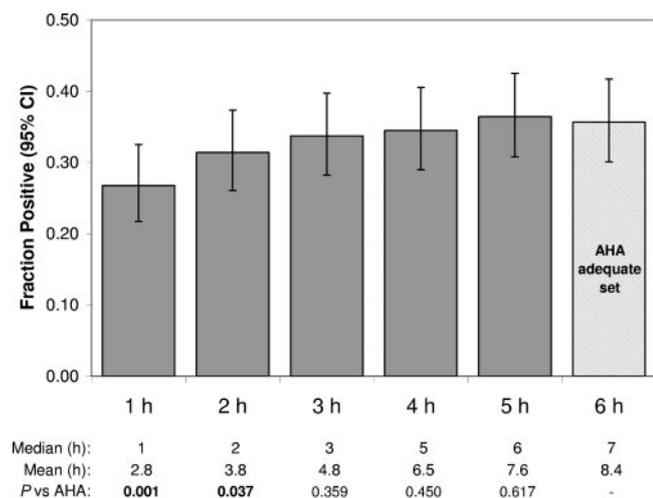

Fig. 1. Effect of decreasing the minimum time interval between specimens on AMI positivity.

AMI positivity [fraction positive with 95% CI (*error bars*)] with progressively shorter minimum intervals (h) between specimens, compared with the 6-h interval in the AHA adequate set (n = 258 for all intervals).

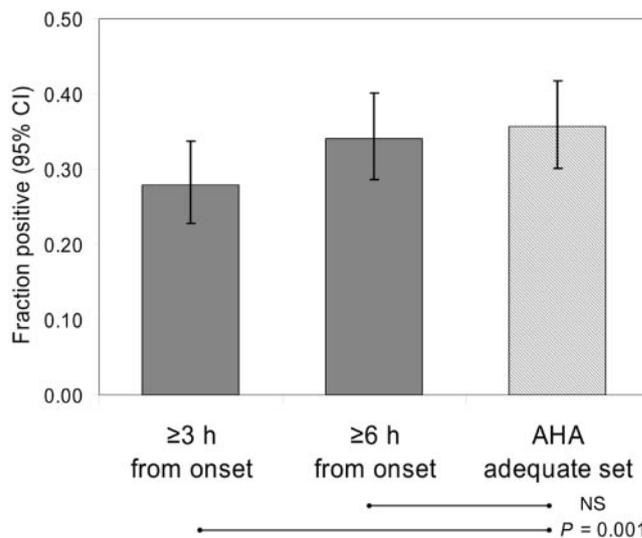|	1 h	2 h	3 h	4 h	5 h	6 h
Median (h):	1	2	3	5	6	7
Mean (h):	2.8	3.8	4.8	6.5	7.6	8.4
P vs AHA:	**0.001**	**0.037**	0.359	0.450	0.617	-

Fig. 2. AMI positivity with the 2 time-from-onset protocols compared with the AHA adequate set.

AMI positivity [fraction positive with 95% CI (*error bars*)] in the 258 patients as determined when at least 1 specimen in the sets was obtained ≥3 h or ≥6 h after the time of onset, compared with the AHA case definition prevalence. *NS*, not significant.

26 patients with STEMI. In contrast, the minimum 3 h from onset protocol identified significantly fewer NSTEMI patients (46 NSTEMI and all 26 STEMI; 72 in total; $P < 0.001$).

When the minimum 6 h from onset protocol and the 6 h from presentation protocol (AHA case definition) were discordant, in all but one case the cTnI concentrations were only minimally increased (Table 2). In the one discordant case with frankly increased cTnI (study number 265; Table 2), the minimum 6 h from onset protocol failed to meet our 20% change criterion; however, this patient with a cTnI concentration > 4 μg/L would not have been missed clinically. In our population, if the minimum 6 h from onset protocol had been used, the diagnosis would have been attained 3.5 h earlier, on average, compared with the AHA adequate specimen set protocol (Fig. 3). An earlier diagnosis was possible in 83% of patients.

Discussion

An adequate set of biomarkers, as defined by the AHA Scientific Statement, requires at least 2 measurements of the same marker taken at least 6 h apart. However, the classification of patients whose markers are collected over a shorter time interval has not been defined previously in an unselected cohort. We assessed whether shorter time intervals between specimens could substitute for the AHA minimum 6-h interval requirement, with a sensitive cTnI assay.
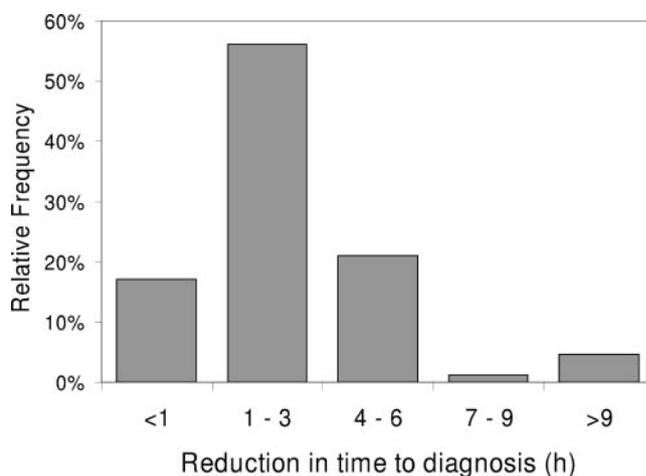
The interval between specimens has 2 purposes: It

Fig. 3. Potential decrease in the time to diagnosis with the ≥6 h from onset protocol.

Patient distribution in terms of the reduction in time (h) to achieve a diagnosis with the ≥6 h from onset protocol compared with the AHA case definition.

reduces the likelihood of testing too early after ischemic cardiac injury (13–15). The interval between specimens is a surrogate, sometimes essential, for reliable knowledge of the time from onset of symptoms of acute ischemic injury. In addition, the interval between specimens allows time for a change in biomarker concentration, which is a requirement for a diagnostic set of biomarkers.

When we examined the effect of reducing the interval between specimens, the positivity of specimen sets in our population was not significantly decreased until the in-

Table 2. cTnI concentrations in all available specimens from the patients who did not conform in positivity between the ≥6 h from onset and AHA specimen set protocols.[a]

Study no.	Positivity ≥6 h onset	AHA	cTnI concentration (μg/L) at time after onset of symptoms																	
			1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h	11 h	12 h	13 h	14 h	15 h	16 h	17 h	18 h	24 h
56	Neg	**Pos**[b]	0.03	**0.05**		0.05	0.04	0.04			**0.06**			0.04							**0.08**
245	**Pos**	Neg		0.01	0.03			**0.06**			0.03			0.01							0.01
249	Neg	**Pos**		0.02	**0.05**	0.05	0.04	0.04			**0.05**			0.03							0.02
82	Neg	**Pos**		0.04	0.04	**0.06**	0.05	0.04			**0.07**			**0.07**							
253	Neg	**Pos**			0.01	0.02	0.03	0.04			**0.05**			**0.06**							0.04
414	Neg	**Pos**			0.00	0.00		0.01			**0.09**			0.00							0.01
321	**Pos**	Neg			0.03	0.03	0.03	**0.05**	0.03	0.04			0.03			0.03					
354	**Pos**	Neg				0.02	0.04	**0.07**			0.03			0.01							
81	**Pos**	Neg				0.01	**0.08**	**0.10**			**0.09**			0.02							
462	Neg	**Pos**				0.02	0.05	0.02			**0.05**			**0.07**							
449	**Pos**	Neg				0.04	**0.05**	**0.05**						0.02							0.03
265	Neg	**Pos**					**3.70**	**4.24**			**7.29**			**8.10**							**9.12**
492	Neg	**Pos**					0.01				0.02			**0.05**							**0.05**
424	Neg	**Pos**						**0.09**			**0.09**			0.05							0.09
456	**Pos**	Neg						**0.11**			**0.15**			**0.12**							
338	Neg	**Pos**										0.07	0.08	**0.09**	0.09		0.09			**0.13**	**0.18**
129	**Pos**	Neg												**0.06**	**0.08**	0.07		0.06	0.07	**0.07**	**0.08**
389	Neg	**Pos**												**0.10**		**0.11**				0.08	**0.12**

[a] From the available specimens, those that are selected by the two protocols are shaded; the presentation specimen is common to both protocols. Positivity was assessed as described in the *Materials and Methods*. Positive results are in bold font.

[b] Pos, positive; Neg, negative.

terval between specimens was less than 3 h. The time distributions of specimen sets under any minimum interval definition will vary from one population to another, affecting diagnostic performance. To compare our population with others, we also examined the performance of an "exact interval" definition (i.e., using only the specimen sets that had exactly the specified time interval). There was no significant difference in performance between these 2 interval definitions, suggesting that the performance of our minimum-interval data was not positively biased by the inclusion of specimen sets with longer intervals, which are likely to perform better in detecting AMI. In our population, 38% of patients presented within 2 h of their reported onset of symptoms. (This fraction was 45% if the 20 patients with unconfirmed onset times were included in the analysis; see the *Materials and Methods*). This prehospital delay time is similar to those in other reports on both American and European populations *(16–18)*.

Most commonly, what caused the AHA-positive patients to become negative at shorter intervals was the absence of an increase in cTnI rather than a failure to detect a concentration change. In our study, 25% of the AMIs that were detected with at least 6 h between specimens were missed when the interval after presentation was decreased to at least 1 h, indicating the need for later samples to detect increases.

An early presentation would lessen the diagnostic potential of cTnI measurements within the first few hours. However, incorporating information about the time of onset into the scheduling of specimen sets would mitigate this effect in early presenters by delaying their second specimen until more time had elapsed from onset. Under this time-from-onset protocol, later presenters could be assessed earlier in their presentation, which would be beneficial. When we included a minimum time from onset in the specimen selections, detection of AMI was maintained when at least one specimen was drawn 6 or more hours after onset. These data are similar to those of Eggers et al. *(4)*, who examined cTnI performance at time of presentation in the subset of their patients presenting >4 h from onset of symptoms. Our study used 2 specimens to assess the change in cTnI concentration. The specimen collection schedule based on 6 h from the time from onset enabled the same rate of detection of AMI as the AHA schedule but achieved this 3.5 h earlier, on average.

There are limitations regarding our findings. The prompt presentation after the onset of symptoms in our population, although probably typical *(16–18)*, is a factor that would diminish the performance of a marker at shorter intervals between specimens. Patients who delay their presentation would be expected to be expressing more diagnostic troponin concentrations at presentation, or any time thereafter, compared with patients who present very early after onset. However, a highly sensitive troponin assay such as the one used in this study might mitigate this effect in early presenters by detecting in-creases earlier during an ischemic event and detecting changes within a shorter interval. Assays with less sensitivity would need to be validated before the shorter protocols examined in the present study could be adopted because troponin assays are not standardized, nor are they of equal sensitivity. In addition, it is clear that the timing of the onset of clinical symptoms can be difficult. In this study, we used estimates provided when the patient was first seen when such data were available. When those data were not available, we used the time of presentation. The critical nature of these data on our analysis cannot be underestimated. It is clear that some patients will be missed if shorter time intervals are used to exclude AMI, but our data suggest that such patients are few in number.

Neither the ESC/ACC nor the AHA case definition specifies criteria for the rising and falling biomarker concentrations required by their definitions for AMI. We chose to implement this aspect of the definition using a 20% difference between the cTnI concentrations in adequate sets of biomarkers. This percentage change was selected because it represents a change that is twice the recommended maximum imprecision (10% CV) for troponin assays *(8)* and is therefore unlikely to be caused by analytical imprecision. Our choice of 20% as the criterion for a rise or fall in concentration is consistent with our other publications on this patient population *(5, 19)* and should be assessed in other population sample sets. This criterion might have missed patients on the slow downslope of the time–concentration curve when minimal changes over 6 h did not meet our 20% criterion *(20)*; however, this effect would be minimal in a population presenting early after onset, as ours did. An absolute concentration change criterion of 0.04 μg/L gave similar performance in this same population *(5, 19)*.

In conclusion, these data indicate that alternative, shorter protocols might be used in epidemiologic and clinical research studies on determining AMI prevalence when the specimen sets do not fulfill the minimum 6-h interval between specimens set forth in the AHA case definition. Using a 1-h interval between specimen pairs, and with at least one specimen collected 6 or more hours from onset, an earlier AMI diagnosis is possible in >80% of patients.

support for lecturing on AMI markers from Roche Diagnostics.

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