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The Sixth Conference on the "Standards of Laboratory Practice Series" sponsored by the National Academy of Clinical Biochemistry (NACB), was held on August 4-5, 1998, at the Annual Meeting of the American Association for Clinical Chemistry, in Chicago, IL. An expert committee was assembled to write recommendations on the use of cardiac markers in coronary artery diseases. The NACB Committee prepared a preliminary draft of the guidelines, made them available on the World Wide Web (www.nacb.org), and distributed them before the presentations. The recommendations were divided into four areas: the use of markers in the triage of patients with chest pain, acute coronary syndromes, clinical applications other than acute myocardial infarction and research, and assay platforms and markers of acute myocardial infarction. The recommendations were revised and subsequently re-presented in part at the "Biomarkers in Acute Cardiac Syndromes Conference", sponsored by the Jewish Hospital Heart and Lung Institute, Louisville KY, on October 16-17, 1998. This report lists each recommendation, its scientific justification, and a summary of discussions from conference participants and reviewers. The majority of this work has been published in *Clinical Chemistry* 1999;45:1104-1121.

Approximately 100 individuals responded to various versions of these recommendations via direct correspondences, telephone calls to Committee members, electronic mail correspondence to the Committee Chairman, or oral questions and comments raised during one of the two conference presentations. Some of the recommendations were changed to reflect the consensus opinion. In cases in which there was no consensus, the Committee included pertinent discussion without necessarily changing the original recommendations. At times, the Committee members felt that although a particular recommendation might not be the current standard of care today, they anticipate that it likely will be adopted in the near future.

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⁷Nonstandard abbreviations: NACB, National Academy of Clinical Biochemistry; ED, emergency department; AMI, acute myocardial infarction; CK and CK-MB, creatine kinase and CK MB isoenzyme; cTnT and cTnI, cardiac troponins T and I; POC, point-of-care; TIMI, Thrombolysis in Myocardial Infarction; and TAT, turnaround time.

⁸Listed with each recommendation is the degree of evidence from the literature and/or agreement from the consensus of participants who attended either presentation. Using a modified classification scheme defined by the American College of Cardiology/American Heart Association (AHA/ACC), the NACB Committee defined a Class I recommendation as one for which there is evidence and/or general agreement; a Class II recommendation as one for which there is conflicting evidence and/or a divergence of opinion about its usefulness/efficacy, but where the weight of evidence/opinion is in its favor; and a Class III recommendation as one for which there is evidence and/or general agreement that a procedure is not useful or effective (12).

Although entitled "Standards of Laboratory Practice," the statements made in this document are "recommendations" and not practice standards. These recommendations represent the individual experiences of experts in the field of clinical biochemistry, cardiology, and emergency medicine, and should be examined for appropriateness in individual or unique settings. These recommendations were authored, in part, to provide education and guidance as to the use of these tests. Discussions contained herein may also stimulate new research studies to be conceived. Members of the discussion panels for the two meetings were as follows (alphabetically): Jesse E. Adams III, Jewish Hospital, Louisville, KY; Eugene Braunwald, Harvard Medical School, Boston, MA; Robert H. Christenson, University of Maryland, Baltimore, MD; Paul O. Collinson, Mayday University Hospital, Surrey, UK; Robert C. Hendel, Northwestern University, Chicago, IL; James W. Hoekstra, Ohio State University, Columbus, OH; Allan S. Jaffe, State University of New York, Syracuse, NY; Hugo A. Katus, Medizinische Universität zu Lubeck, Lubeck, Germany; Jack H. Ladenson, Washington University, St. Louis, MO; E. Magnus Ohman, Duke University, Durham, NC; David B. Sacks, Brigham & Women's Hospital, Boston, MA; and Michael H. Salinger, Evanston Northwestern Healthcare, Evanston, IL. Mauro Panteghini, Brescia, Italy (Chair) and Francesco Dati, DiaSys Diagnostics, Holzheim, Germany also participated in discussions of these recommendations as members of the International Federation of Clinical Chemistry Committee for the Standardization of Markers of Cardiac Damage.

Session I. Recommendations for Markers in the Triage of Patients with Chest Pain

Introduction to Section I

Coronary artery disease remains today as the leading cause of morbidity and mortality throughout the western world. In the U.S. alone, 8 million out of the total of 95 million annual visits to the emergency department (ED)⁷ is for a presentation of acute chest pain (Figure 1)(1). Of this total, 5 million are suspected of acute cardiac disease. The annual incidence of unstable angina (UA) and acute myocardial infarction (AMI) is 1.2 and 1.0 million, respectively. Sudden acute cardiac death occurs in about 300 thousand patients, while the remainder have a non-cardiac cause of chest pain and are discharged from the ED. The differential diagnosis of acute chest pain is summarized in Table 1. Several of the diagnoses listed have a low probability of being the etiology of the chest pain. Nevertheless, the ED physician must rule them out, as many carry a significant potential for producing morbidity and mortality.

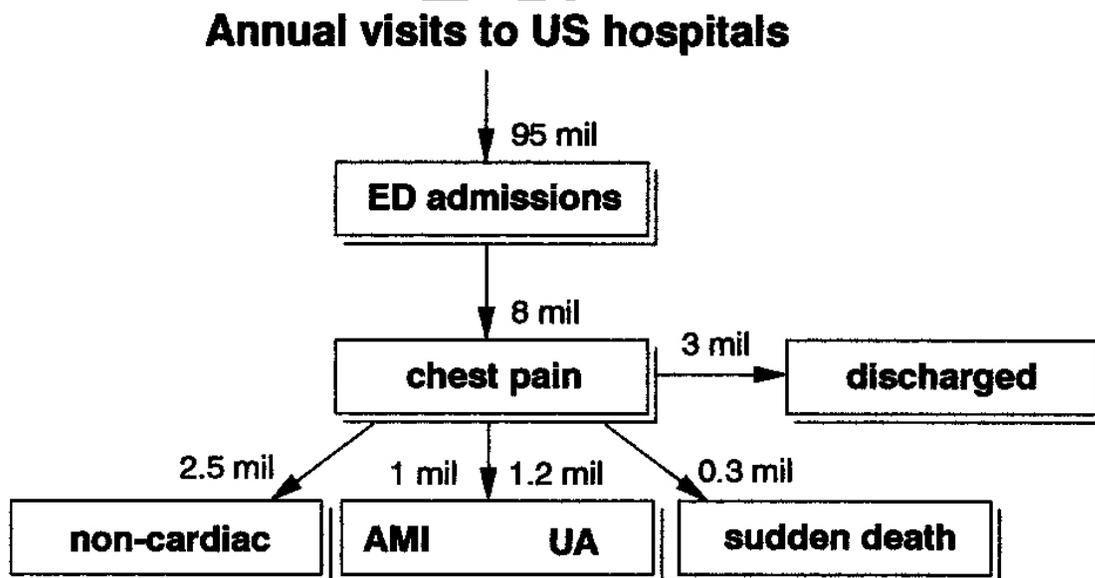


Fig.

1. Demographics and outcomes of patients who present to emergency departments in the U.S. with chest pain.

Table 1. Common cause of chest pain.^a

Cardiac	Pulmonary	Others
<i>Ischemic syndromes</i>	Bronchitis	<i>Vascular</i>
Stable angina	Bronchospasm	Aortic dissection
Unstable angina	Empyema	Pulmonary embolism
Variant angina	Pleural effusion	Pulmonary hypertension
AMI	Pleuritis	<i>Gastrointestinal</i>
<i>Valvular disease</i>	Pneumonia	Esophageal spasm
Mitral valve prolapse	Pneumothorax	Gastroesophageal reflux
Aortic stenosis	Pulmonary edema	Mallory-Weiss tear
Subaortic stenosis	Aortic dissection	Esophagitis/gastritis
<i>Cardiomyopathy</i>	Pulmonary embolism	Gastric/duodenal ulcer
<i>Pericarditis</i>	Pulmonary hypertension	Biliary colic
		<i>Musculoskeletal</i>
		Costochondritis
		Muscle strain/spasm
		Cervical radiculopathy
		<i>Neurologic</i>
		Herpes Zoster

^aTaken from Green GB, Green SF. Markers of myocardial injury in the evaluation of the emergency department patient with chest pain. In: Wu et al. ed., Cardiac Markers, Totowa NJ: Humana Press, 1998, p. 77.

Recommendation 1

The triage of patients with chest pain from the emergency department is one of the most difficult challenges that face ED physicians today. Admission of patients with a low probability of acute coronary artery disease often leads to excessive hospital costs (2). A strategy that is too liberal with regard to ED discharges may lead to higher numbers of patients released with acute myocardial infarction (AMI). As summarized in Table 2, inappropriate discharge of ED patients who have AMI has been estimated to occur in 2-13% of patients and is the single most common cause of malpractice lawsuits against ED physicians today (3-11).

Table 2. Rate of inappropriate discharge from the ED for patients with AMI

Study	Year	Percentage
Pozen et al. (5)	1984	7%
Tierney et al. (6)	1986	13%
Lee et al. (7)	1987	4%
Rouan et al. (8)	1987	10%
McCarthy et al. (9)	1993	2%
Puleo et al. (10)	1994	5%
Graff et al. (11) 1997		4.5%

Recommendation: Members of emergency departments, divisions of cardiology, hospital administration, and clinical laboratories should work collectively to develop an accelerated protocol for use of biochemical markers for the evaluation of patients with possible acute coronary syndromes (ACS).

Strength/consensus of recommendation: Class I.⁸

For simplicity, this protocol should apply to either the facilitated diagnosis or the rule-out of AMI in the ED or to routine diagnosis from other areas of the hospital, should a patient develop symptoms consistent with acute coronary syndromes while hospitalized.

Strength/consensus of recommendation: Class II.

Many hospitals today have a dedicated area within the ED for the rapid rule-out of AMI. These areas have been designated as "chest pain centers", "heart emergency rooms", or some other terms to indicate that the efficient triage of chest pain patients is a major objective of that center. Figure 2 lists the necessary elements of a chest pain center. Essential for early AMI rule-out is frequent electrocardiographic (ECG) testing and blood collections for the measurement of cardiac markers. Patients with negative results for these tests most likely do not have an AMI. They may, however, have unstable angina or other forms of acute cardiovascular disease. For these patients, it is

appropriate to perform additional studies such as a stress test, echocardiogram, or radionuclide ventriculogram for risk stratification. Establishment of a clinical practice guideline for the evaluation of patients with chest pain will reduce the variability of practices among physicians and institutions, at the same time improving the accuracy of triaging decisions (13). The NACB Committee felt that for "routine AMI diagnosis" of patients who are already hospitalized for other reasons, the same principles and criteria should apply as are used in the ED.

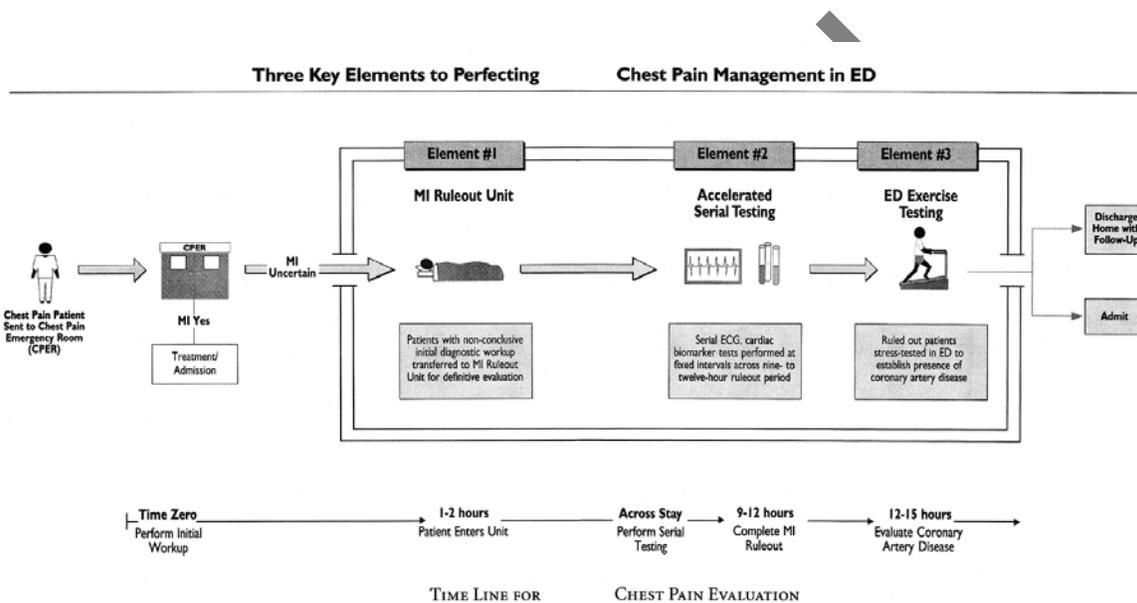


Fig. 2 Time line for chest pain evaluation centers. From perfecting MI Ruleout. Best Practices for emergency evaluation of chest pain. Cardiology Preeminence Roundtable, Wahington, DC, 1994, used with permission.

Discussion

Although the recommendation that laboratorians should work with ED physicians, cardiologists, and hospital administration may appear obvious, in actual practice, decisions on testing protocols are often made without input from the laboratory. Laboratory directors must be aggressive in requesting that qualified personnel be part of organizational and operating committees when such discussions are being conducted,

or should initiate the discussions themselves. Understanding the expanded role that the laboratory will play in creating these rule-out centers will enable justification to hospital administrators for the additional laboratory expenses that will be required. This argument will be particularly effective if the overall objective of reducing in-hospital lengths of stay and the numbers of unnecessary admissions or wrongful discharges from the ED can be demonstrated.

The diagnosis of AMI is not always made in the ED. Sometimes patients admitted for other reasons develop symptoms for AMI while in the hospital. Some physicians or administrators may believe that rapid AMI rule-out of hospitalized patients may not be as important as triage for ED patients. Nevertheless, the NACB Committee felt that the same protocol used in the ED is appropriate for routine AMI diagnosis because new therapies for acute coronary syndromes are available, and, when appropriate, should be delivered rapidly. The use of a rapid AMI rule-out protocol will simplify the steps needed from the laboratory's perspective and provide clinicians optimum diagnostic measures for all patients.

Recommendation 2

Although the time of onset of chest pain for AMI patients is often known, this information often is less available or reliable for those with unstable angina and other cardiac diseases. It is not uncommon for these patients to report multiple episodes of chest pain over the hours and days before ED presentation. Intermittent closure and spontaneous reperfusion of coronary arteries with ruptured atherosclerotic plaques reflect the dynamic nature of acute coronary syndromes. In the elderly or in patients with insulin-dependent diabetes mellitus type I, there may be altered thresholds or a blunted response to pain. Indeed, there are many patients with acute coronary syndromes who experience silent ischemia and infarction (i.e., no pain during occlusive episodes) (14).

Recommendation: For routine clinical practice, blood collections should be referenced relative to the time of presentation to the ED and (when available) the reported time of chest pain onset.

Strength/consensus of recommendation: Class I.

Discussion

In the early drafts of the Guidelines, the recommendations were that all blood collections should be referenced to the time of ED presentation only. However, many reviewers felt it important to also note the time of onset of chest pain, especially when there is a history of a single chest pain event (and not several events over many days) and when the time of onset as reported by the patient or family is deemed to be reliable. It may also provide an explanation as to why some clinical studies fail to document a consistent rise in the concentration of the marker, e.g., at 6 h, whereas other studies indicate that the markers were increased at this time point in all patients (e.g., when the majority of enrolled patients in the study present beyond 6 h of chest pain).

Recommendation 3

The ideal biochemical marker would be one that has high clinical sensitivity and specificity, appears early after AMI to facilitate early diagnosis, remains abnormal for several days after AMI, and can be assayed with a rapid turnaround time (15,16). Because there currently is no single marker that meets all of these criteria, a multianalyte approach has the most merit.

Because the interval between the onset of pain and ED presentation is variable from patient to patient, two markers are needed to enable detection of patients who present either early or late. Currently, myoglobin is the marker that most effectively fits the role as an early marker. A rise in myoglobin is detectable in blood as early as 1-2 h

after onset and can be highly effective for AMI rule-out (Fig. 3, peak A)(17). Moreover, automated immunoassays for myoglobin are commercially available. Myoglobin is not cardiac specific, and patients with renal failure, skeletal muscle injury, trauma, or disease can have abnormal concentrations in the absence of AMI (18). The creatine kinase MB (CK-MB) isoforms (also termed "subforms") have also been shown to be an early marker for AMI (10). Automated stat CK-MB isoform measurements are being used in some hospitals as an early measure of myocardial injury. Moreover, it may also be possible that troponin can be used as an early marker if new assays are developed that are more sensitive than current ones (19). In an ED study, qualitative measurement of cardiac troponin T and I (cTnT and cTnI) using point-of-care (POC) devices were reliable for ruling out AMI at 6 h after onset of symptoms (20). These studies, however, were not confirmed by a more recent study of chest pain patients that used quantitative laboratory-based assays for troponin (21). Clearly, more studies are needed to fully address the role of troponin T and I in early diagnosis.

In contrast, cTnT and cTnI are currently the best markers for definitive AMI diagnosis. Troponins appear in the serum relatively early after the onset of symptoms (4-12 h) and remain abnormal for 4-10 days (Fig. 3, peak B). Results are not increased in the presence of skeletal muscle troponin (22,23). Early studies have questioned the clinical specificity of cTnT assays in patients with chronic renal failure (24,25). With the development of a second- and third-generation ELISA assay for cTnT, the frequency of positive results in these patients is lower than the frequency in the first-generation assay, although still higher than for cTnI (26,27). Western blot analysis on regenerating human skeletal muscle tissue showed that the cardiac isoforms of troponin T are expressed in pathologic conditions (such as renal disease, polymyositis and muscular dystrophy) (28). However, subsequent studies have shown that the antibodies used in the Roche commercial assays are specific for myocardial cTnT isoforms, do not detect

the cTnT isoforms expressed in diseased skeletal muscle, and therefore, do not produce false-positive cTnT results in renal patients (29,30).

Preliminary outcomes studies have shown that chronic renal failure patients who have high cTnT concentrations in blood have a higher incidence of cardiac death than those with normal concentrations, confirming the notion that troponin is measuring true myocardial injury that is not associated with or classified as an AMI (31). The importance of these findings is not completely known. Are there therapies that can be administered to reduce the short-term mortality of renal failure patients with a positive troponin result? How does risk stratification with troponin compare with other indicators of renal function? One study showed that measurement of the troponins in patients with both acute coronary syndromes and renal insufficiencies reduces the effectiveness for risk stratification of chest pain patients based on cTnT and cTnI monitoring (32). As more studies are completed in this area, a meta-analysis may be necessary to resolve these continuing issues.

Historical

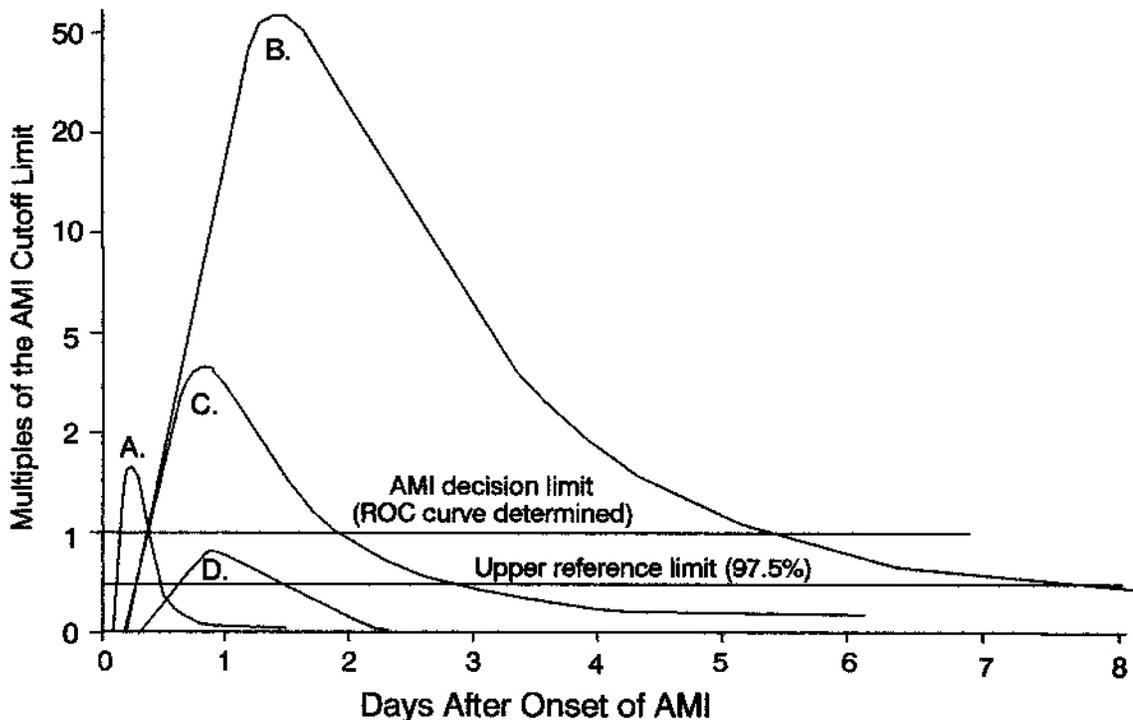


Fig. 3. Plot of the appearance of cardiac markers in blood vs time after onset of symptoms. Peak A, early release of myoglobin or CKMB isoforms after AMI; peak B, cardiac troponin after AMI; peak C, CK-MB after AMI; peak D, cardiac troponin after unstable angina. Data are plotted on a relative scale, where 1.0 is set at the AMI cutoff concentration.

Recommendation: Two biochemical markers should be used for routine AMI diagnosis: an early marker (reliably increased in blood within 6 h after onset of symptoms) and a definitive marker (increased in blood after 6-9 hours but has high sensitivity and specificity for myocardial injury, remaining abnormal for several days after onset).

Strength/consensus of recommendation: Class II.

Discussion

The merits of myoglobin, as the early marker, have been debated by many reviewers and conference participants. Although there is ample literature suggesting that myoglobin is an early marker (33-35), there are reports that support the view that

myoglobin is not any earlier than CK-MB mass assays (36). These critics feel that the poor specificity of myoglobin (in the presence of skeletal muscle disease or renal failure) does not justify its routine use as a cardiac marker. However, there is increasing pressure by ED physicians and hospital administrators to rule out AMI sooner. Some chest pain centers have begun to discharge patients within 6 h of ED presentation. CK-MB is not reliably increased at this interval after AMI, and myoglobin may have a role in this situation. As an alternative to myoglobin, a minority of laboratories have begun using CK-MB isoforms as an early AMI marker (21,37). (In a poll taken during the AACC Annual Meeting, <1% of conference participants indicated that they were currently using isoforms.) Currently, CK-MB isoforms are most effectively measured by high-voltage electrophoresis (38). With improvements in analytical methodologies, the number of laboratories routinely using isoforms might increase. The NACB Committee recognizes the limitations of myoglobin and CK-MB isoforms and encourages continued research into earlier markers, particularly if they are more specific for myocardial necrosis. In the meantime, the NACB Committee believes that myoglobin is an earlier marker than CK-MB mass and is more conveniently measured on automated immunoassay analyzers than CK-MB isoforms.

Recommendation 4

Large studies in New York and Texas have shown that about 50% of AMI patients will present to the ED with evidence of acute myocardial infarction on the electrocardiogram (ECG) (39). Acute intervention with thrombolytic therapy or angioplasty should be considered in those patients who present within 12 h after the onset of symptoms (40,41). Specific ECG changes are highly diagnostic for AMI when interpreted by well-trained physicians (42).

Recommendation: In patients with a diagnostic ECG on presentation (ST-segment elevations, presence of Q waves or left bundle branch block in two or more contiguous leads), the diagnosis of AMI can be made and acute treatment initiated without results of acute cardiac marker testing.

Strength/consensus of recommendation: Class I.

In AMI patients with diagnostic ECGs, biochemical marker testing at a reduced frequency of blood collection (e.g., twice per day) is valuable for confirmation of diagnosis, to qualitatively estimate the size of the infarction, and to detect the presence of complications such as a reinfarction.

Strength/consensus of recommendation: Class I.

Discussion

The NACB Committee sought advice from ED physicians and cardiologists as to why cardiac markers are still being ordered on patients with ECG-documented AMI, when in many cases, therapy had already been initiated before results of tests were available from the laboratory. Although most physicians recognize that in this context, these tests do not serve a diagnostic role, many felt that biochemical documentation of AMI was necessary to complete the triad of criteria established by WHO for AMI diagnosis (43). It is also likely that a positive result for a cardiac marker in these patients provided a level of comfort and confidence to the attending staff. Many physicians also felt that knowing the peak concentration of a cardiac marker provided a qualitative estimate of infarct size (without calculating the area under the curve of marker

concentration vs time). This information might have a role in the future management of surviving AMI patients.

Many conference participants also felt that continued measurement of markers was helpful in detecting the presence of a reinfarction, estimated to be 17% of AMI patients (44). If the reinfarction occurs before there is complete clearance of the marker from the original infarct, it might not be possible to detect the presence of the reinfarction because the markers released from the second event might be indistinguishable from that released by the initial event. For this reason, the use of cardiac markers that return to baseline concentrations early may have an advantage over the use of markers that are slow to clear from the circulation. For example, myoglobin and CK-MB isoforms return to reference values typically within 24 h after AMI (Fig. 3, peak A). If a reinfarction were to occur after this time, increases in the concentration or activity of these proteins would enable detection of a second necrotic event. CK-MB mass can also be considered as a reinfarction marker that returns to baseline concentration reasonably early (but not as early as myoglobin). Many reinfarctions occur between 7 and 14 days after the initial event. Because CK-MB remains abnormal for 3-4 days (Fig. 3, peak C), CK-MB may be useful to detect a reinfarction even if the event is not immediately suspected by the medical staff. CK-MB mass would show a secondary increase, whereas myoglobin and CK-MB isoforms could have returned to baseline concentrations (Fig. 3, peak A). Alternatively, one could request that the laboratory retrieve a stored specimen for myoglobin or isoform testing if available because serum myoglobin is stable for several days if refrigerated (45), and isoforms are stable when collected with EDTA (46).

Recommendation 5

For AMI rule-out of patients who have equivocal ECG changes, cardiac markers play an essential diagnostic role in non-Q-wave AMIs. Unfortunately, there is great

variability between hospitals in the frequency of blood collections. In 1986, the American College of Physicians recommended a conservative testing guideline based on total CK and CK-MB for blood collected on admission and at 12 and 24 h after admission, and the use of lactate dehydrogenase isoenzymes when admission is >24 h after onset (47). The NACB Committee believes that this strategy is no longer adequate to meet the current triaging needs.

Rule-out of AMI requires serial collection and testing of blood for cardiac markers. When an early marker such as myoglobin is used, acute myocardial necrosis can be effectively ruled out within 6-9 h after ED presentation (48,49), and a decision to discharge the patient to home or a to low care level bed can be considered. On the other hand, for AMI rule-in, a single positive result for either troponin T or I would trigger a diagnosis of AMI and triage of the patient to the appropriate level of care (14), without the need for necessarily completing this algorithm (50,51). This recommendation was made because, unlike myoglobin, CK, CK-MB, and lactate dehydrogenase, positive results for cTnT and cTnI are highly indicative of myocardial damage, with no release of these proteins from skeletal muscles or other tissues (52,53).

Recommendation: For detection of AMI by enzyme or protein markers, in the absence of definitive ECGs, the following sampling frequency is recommended:

<u>marker</u>	<u>admission</u>	<u>2-4 h</u>	<u>6-9 h</u>	<u>12-24 h</u>
early (<6 h)	x	x	x	(x)
late (>6 h)	x	x	x	(x)

(x) indicates optional determinations.

Strength/consensus of recommendation: Class II.

Discussion

The need to perform the 2-4 h blood collection for the late marker can be questioned. In particular, negative results at admission and at 2-4 h after admission for myoglobin, and a negative result for cardiac troponin at admission would obviate the need for measuring troponin in the 2-4 h sample. The NACB Committee felt that most laboratories do not currently have a mechanism for automatic "reflex testing" (i.e., testing that involves the ordering or cancellation of follow-up tests on a given sample based on results of preliminary tests). Therefore, it is more convenient for the laboratory to perform testing for both markers on all samples, rather than to hold specimens until results of preliminary tests (i.e., the early markers) are known.

Among chest pain centers, there are many variations to the protocol for blood sampling and the total number of samples needed for AMI rule-out. Some centers use intervals of every 3 h, whereas others use every 4 h. In one study using POC devices, chest pain patients were triaged on the basis of only two samples collected: one at admission and one at 4 h (20), with a third sample collected only on patients presenting with a 2 h history of chest pain. Because of the unreliability of the chest pain history, the NACB Committee has taken a more conservative approach of recommending the collection of at least three blood samples during the early triage period. A blood collection at 12-24 h may be useful for the detection of reinfarction or myocardial extension or for risk stratification of patients with unstable angina. Investigators have found that a 16-h blood sample adds additional value for risk stratification over the initial blood sample (54).

Recommendation 6

Some EDs have not yet developed a rapid rule-out chest pain center because of financial limitations, space, and/or a lack of knowledge of the potential benefits. In these centers, the extra laboratory tests bring additional costs without benefits in terms of

reduced hospital lengths of stay or frequency of inappropriate discharges of patients with AMI.

Recommendation: For those Eds in which patient triage decisions are not made within the first few hours after ED presentation, the use of an early marker such as myoglobin may be unnecessary. In this case, only one definitive marker such as cardiac troponin is needed. The frequency of blood collection should also be reduced.

Strength/consensus of recommendation: Class I.

Historical

Session II. Recommendations for Markers in Acute Coronary Syndromes

Introduction to Section II

1. The acute coronary syndrome (ACS) is a pathophysiologic continuum that results from rupture of an atherosclerotic plaque and an associated thrombus (55). It can ultimately result in clinical presentations ranging from entirely asymptomatic to unstable angina to massive acute myocardial infarction. ACS is the culmination of a series of events that begins with atherosclerosis, the narrowing of coronary arteries by deposition of highly lipid-filled plaque. (The etiologies, risk factors, and laboratory markers for atherosclerosis (lipoprotein metabolism, coagulation factors, genetics, etc) are complex and beyond the scope of this monograph.) The American Heart Association have subdivided the plaque progression into distinct phases (56). Plaques formed in the early phases (I-III) are stable in that they have a thick fibrous cap, are not at risk for rupture, and the patient experiences no cardiac symptoms. However, for many patients, the plaque progresses to phases IV and Va which are lesions characterized by the thinning of the cap and are vulnerable to rupture (Figure 4A). The fibrous cap can be thinned by inflammation and monocyte infiltration, activation of metalloproteinases, oxidation of LDL lipoproteins, augmentation of growth factors, and other processes. Shear stresses from diastolic blood pressure can lead to plaque ruptures in vulnerable areas such as the edges or shoulders of plaque lesions or bifurcations of the arterial tree (Figure 4B). The exposure of the core contents of lipids, cellular and extracellular elements (collectively termed “gruel”) results in thrombus formation and platelet aggregation, and the development of chest pain (Figure 4C). Incomplete occlusion of the coronary artery leads to unstable angina, while total occlusion lead to AMI (Figure 4D). Figure 5 summarizes the events and potential markers for each event that takes place after plaque rupture in acute coronary syndromes.

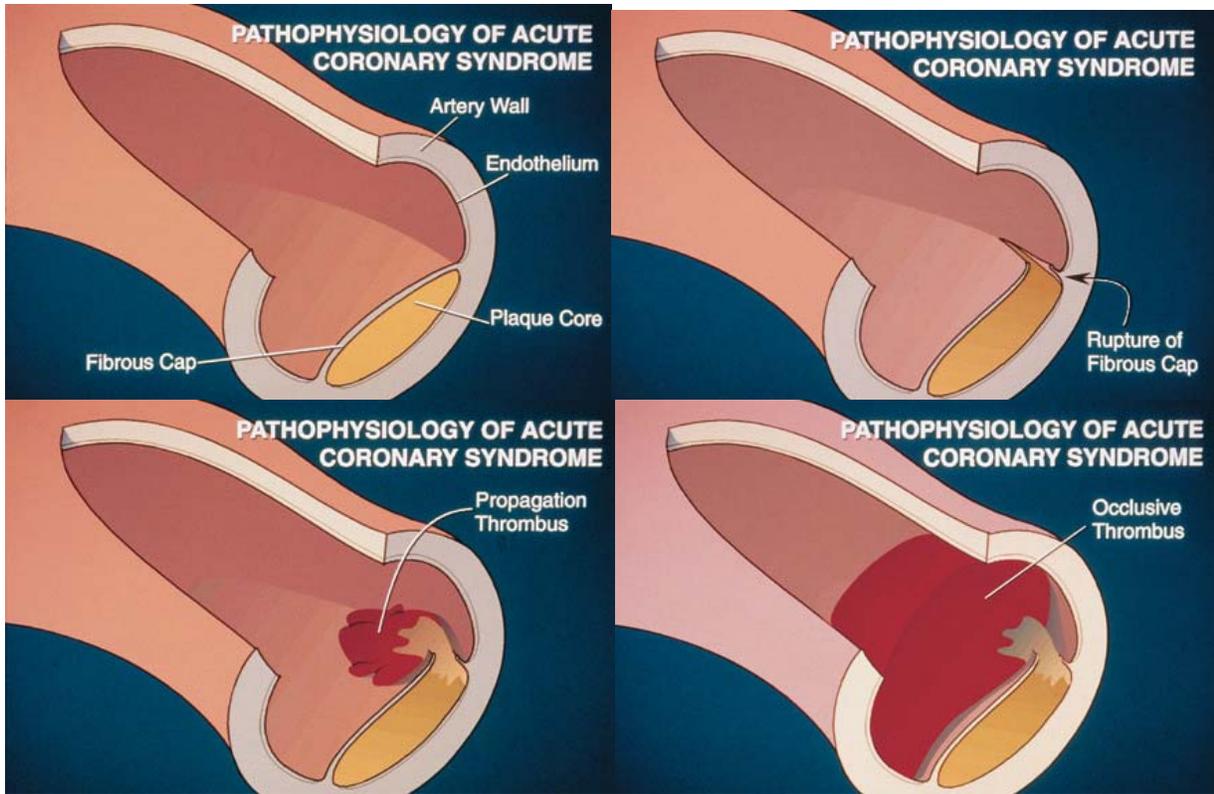


Fig. 4. Pathophysiology of acute coronary syndromes. A. Cross-section of coronary artery showing the presence of a lipid-filled plaque with a thin fibrous cap. B. Rupture occurring at the shoulder region of the plaque, which is an area of vulnerability due to high circulatory shear stress. C. Exposure of plaque core elements propagates thrombus formation. D. Totally occlusive thrombus causing AMI. Reprint from *Clinical Laboratory News*, Jun 1998, page 12-14, with permission from the American Association for Clinical Chemistry.

Pathophysiology of Acute Coronary Syndrome

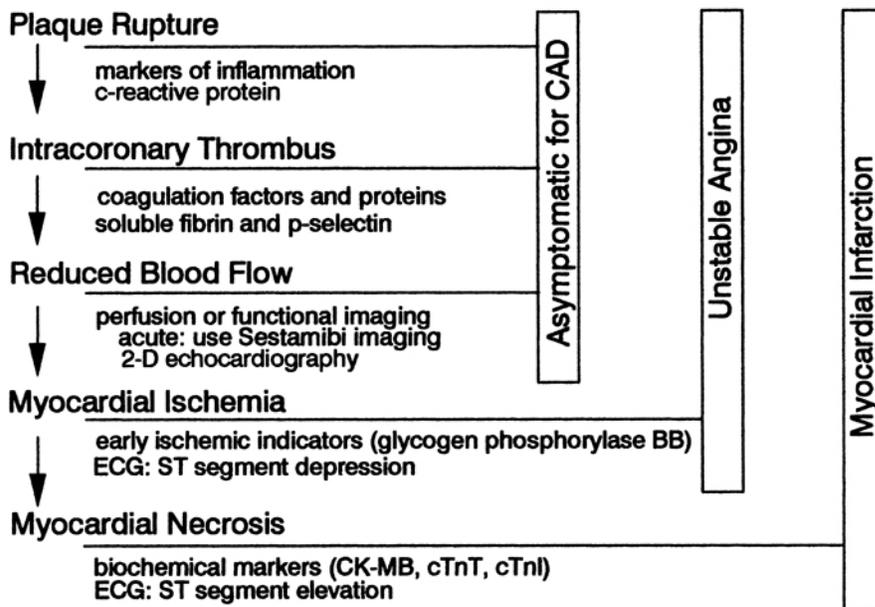


Fig. 5. Summary of pathophysiologic events in acute coronary syndromes. Reprint from *Clinical Laboratory News*, Jun 1996, poster insert, with permission from the American Association for Clinical Chemistry.

Recommendation 1

The acute coronary syndrome is a pathophysiologic continuum that results from rupture of an atherosclerotic plaque, with subsequent platelet aggregation and thrombus formation (57,58). It can lead to clinical presentations ranging from entirely asymptomatic to unstable angina to AMI to sudden cardiac death attributable to arrhythmias. There have been major improvements in the specificity of new cardiac markers (such as cardiac troponin) and increases in analytical sensitivity for older markers such as CK-MB. When improved markers are compared to accepted standard markers, such as CK-MB, results that are discordant to each other can occur. For example, what does a positive troponin in a chest pain patient suggest when CK-MB is within the health-related reference interval? With improvements in the analytical

sensitivity of these assays, it is now evident that small increases in sensitive markers such as cardiac troponin provide additional clinical information that is not evident with conventional enzyme markers.

Original validation studies for cardiac troponin assays have compared results against CK-MB for the diagnosis of AMI. When the upper limit of normal is used as the troponin cutoff concentration, clinical studies have shown that cardiac troponin was less specific for AMI diagnosis than CK-MB mass (59), using the classical WHO definition of AMI (43). This was because assays for cardiac troponin were detecting myocardial injury in some cardiac patients (e.g., those with unstable angina) with CK-MB below the cutoff (Fig. 3, peak C), and the extent of damage was insufficient to produce ECG patterns that were indicative of AMI. A higher troponin cutoff concentration could be used to mimic the clinical specificity of CK-MB for AMI. However, this choice will lead to the loss of clinically useful information because the importance of detecting myocardial injury (Fig. 3, peak D) has been demonstrated in retrospective outcomes studies in patients with abnormal concentrations of cTnT (60-62) or cTnI (63-65).

These studies define a population that is at high short-term risk (6 weeks) for adverse events (AMI and cardiac death). Cumulative meta-analyses suggest that the odds ratio for adverse events of a high troponin in unstable angina are 5:1 relative to a cohort of chest pain patients with normal troponin results (66). The risk is additive: the higher the cTnT and cTnI concentrations in blood, the higher the prospective risk (65,67). Thus, the detection of a low degree of myocardial injury is possible with the use of a low cutoff concentration for cardiac troponin (e.g., the upper limit of the reference interval), a strategy that is less applicable for nonspecific markers such as CK-MB.

The methodology for assignment of the low and high cutoff concentrations for cardiac troponin or any other cardiac marker is discussed in Session III under "Recommendation 5."

Recommendation: Two decision limits are needed for the optimum use of sensitive and specific cardiac markers such as cTnT or cTnI. A low abnormal value establishes the first presence of true myocardial injury, and a higher value is suggestive of injury to the extent that it qualifies as AMI, as defined previously by WHO (36).

Strength/consensus of recommendation: Class II.

Discussion

The concept of two decision limits for cardiac troponin was highly debated during the presentation of the Guidelines. A survey indicated that slightly more participants would prefer the use of a single cutoff concentration set at the lower of the two decision limits, rather than define two separate limits. No one suggested the use of a single cardiac troponin decision limit set at the AMI cutoff concentration. Many felt that the use of two limits overly complicates the situation and would require a substantial amount of physician education. Others felt that the therapeutic approaches for patients with unstable angina and non-Q-wave AMI are identical and that a differentiation between these two groups is, therefore, unnecessary.

The NACB Committee agreed with the consensus that detection of any myocardial injury was important (60), thereby justifying the use of a single low cutoff concentration for cardiac troponin. However, the Committee felt that use of a more sensitive cardiac marker (in a patient with a positive history of chest pain) would double the number cases of AMI compared with using the existing WHO criteria, which are based on the use of enzyme markers. It is important to not classify these patients as AMI, because they may be disadvantaged from a social, psychological, and socioeconomic standpoint (68). It may also affect how the hospital gets reimbursed for these services. Until the criteria for diagnosis of AMI are redefined by WHO or other

clinical groups such as the American Heart Association or the American College of Cardiology, the NACB Committee recommends a two-cutoff designation for cardiac troponin; a low limit that detects a small amount of myocardial injury but classifies those patients at high risk, and a higher limit with the amount of injury present is to the extent that it conforms with a WHO-defined AMI. Figure 6 summarizes the selection of one cutoff concentration for a nonspecific biochemical marker such as CK-MB, and two cutoff concentrations for use of a specific biochemical marker such as the troponins (69).

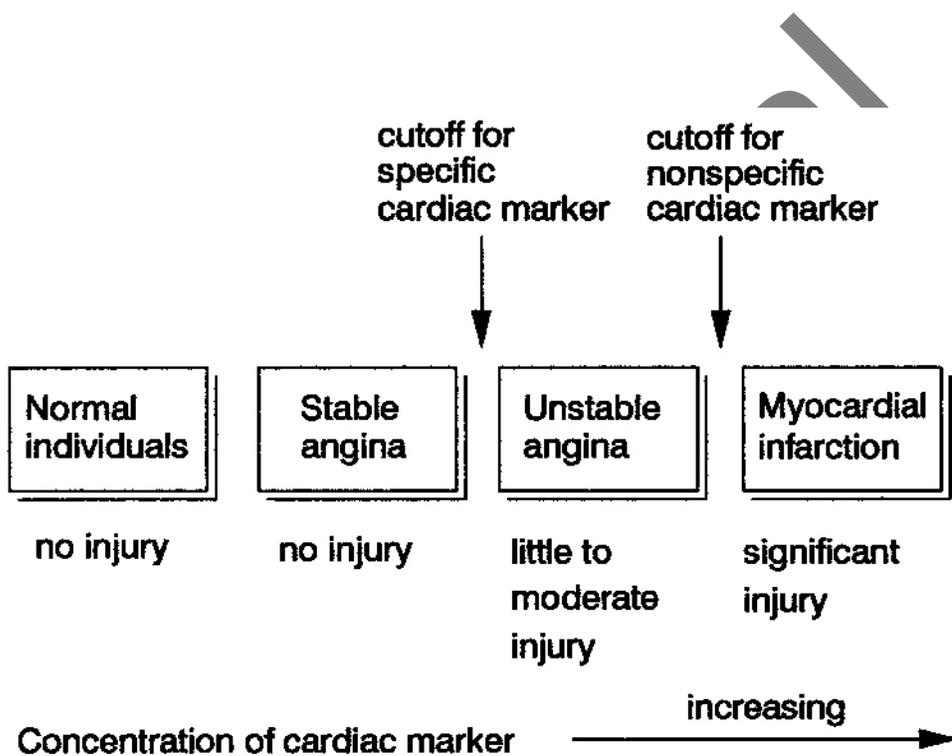


Fig. 6. Cutoff concentration for use of a non-specific marker such as CK-MB have traditionally been set to differentiate between patients with unstable angina and AMI. Use of a biochemical marker that is highly specific for cardiac injury enables the selection of two cutoff concentrations: differentiation between unstable angina vs. AMI, and stable angina vs. unstable angina. Used with permission from Wu AHB, Clin Chim Acta 1998;272:11-21.

Recommendation 2

In the past, CK-MB results between the upper limit of normal and the AMI decision limits had been termed the "gray zone." This practice was appropriate because CK-MB was not specific for the heart, and there were healthy subjects who had

measurable CK-MB concentrations from skeletal muscle release within this range. The use of a low CK-MB cutoff would cause many of these patients to be incorrectly classified as having high cardiac risk. For cTnT and cTnI, the term gray zone should not be used because it connotes uncertainty in the clinical interpretation.

Recommendation: Chest pain patients with laboratory results for cTnT and cTnI between the upper limit of the reference interval and the decision limit for AMI should be labeled as having "myocardial injury." These patients should be admitted and acutely treated to reduce the risks associated with this injury (60,61).

Strength/consensus of recommendation: Class I.

Discussion

In the original draft of these Recommendations and in some early literature reports on cardiac troponin [e.g., Ref. 72], abnormal troponin results occurring in some non-AMI patients with CK-MB within the reference interval were designated as having "minor myocardial injury or damage." The descriptive term, "minor" meant that the amount of tissue damage occurring to the heart was significantly less than that which occurs in patients with AMI. However, many conference participants felt that use of this term might be interpreted by physicians as minor risk for future untoward cardiac events, which is not true. In fact, unstable angina patients with abnormal concentrations of troponin may be at greater risk than surviving AMI patients because therapeutic options such as intravenous thrombolytic therapy are not available for the non-AMI patient. Other terms have been suggested that might better describe the clinical importance of this finding, such as "microinfarct" or "infarctlet," or suggest that these patients have suffered a non-Q-wave AMI (73). Perhaps in some future clinical guideline, the term "acute myocardial infarction" can be eliminated entirely and replaced

with "acute coronary syndromes." In this way, a single cutoff concentration for a cardiac marker such as troponin can be justified. This would reflect the incremental risks associated with increasing concentrations of the marker, consistent with the continuous injury concept of acute coronary syndromes.

In the current version of these Guidelines, the term minor has been removed. Excluding situations where the cardiac troponin was increased because of a problem with the assay's analytical specificity, all patients with an abnormal concentration of troponin have myocardial injury and should be viewed as having cardiovascular risk. It is the responsibility of the ordering physician to use this information in the context of other data in making the appropriate management decision.

It is also important to recognize that because troponin is increased for many days after AMI, it may be possible that without a full clinical history, small increases in troponin with a negative CK-MB might simply reflect an AMI in which CK-MB had returned to normal. Because of this fact, some might advocate keeping CK-MB mass assays available for this purpose. However, myoglobin could also fulfill this need because it would be normal in these late-presenting AMI patients. Myoglobin would be available if the recommendations for two cardiac markers for ED triaging were followed by an institution.

Recommendation 3

WHO has defined the diagnosis of AMI as a triad (43). Two of which must be present for diagnosis:

- i. The history is typical if severe and prolonged chest pain is present;
- ii. Unequivocal ECG changes that are the development of abnormal, persistent Q or QS waves, and evolving injury lasting longer than 1 day; and

- iii. Unequivocal change consisting of serial enzyme changes, or initial rise and subsequent fall. The changes must be properly related to the particular enzyme and to the delay time between the onset of symptoms and blood sampling.

With the development of biochemical markers that are not themselves enzymes, such as cTnT, cTnI, and myoglobin, the third criterion of the WHO triad should be revised.

Recommendation: The WHO definition of AMI should be expanded to include the use of serial biochemical markers and not be limited to enzyme changes. It should be emphasized that rule-out of AMI cannot be made on the basis of data from a single blood collection. However, when very specific cardiac markers are used, the presence of an abnormal concentration from a single specimen can be highly diagnostic of myocardial injury.

Strength/consensus of recommendation: Class I.

Discussion

The NACB Committee recognizes that clinical groups will have to lobby WHO to make substantive changes to their criteria for AMI diagnosis. This will require an international effort by cardiologists, emergency physicians, and laboratorians. Thus, the above recommendation is included to justify the use of myoglobin and cardiac troponin, and perhaps future non-enzyme protein markers that will have been shown to have value in the diagnosis of AMI.

Recommendation 4

The analysis of blood for lipids such as cholesterol and lipoproteins such as LDL and HDL is well established in the assessment of coronary artery disease risk (74). As such, these markers are being used to screen asymptomatic individuals. Because sensitive cardiac markers have also been shown to provide information on risk stratification, there may be an impetus to use these markers as part of a biochemical panel for routine health screening to detect the presence of silent ischemia, or after exercise stress testing to detect presence of ischemic injury. Studies of biochemical markers before and after nuclear ventriculography of chest pain patients have shown that neither cTnT or cTnI is increased after stress testing, even in patients with documented evidence of flow defects (75).

Recommendation: At this time, there are no data available to recommend use of cardiac markers such as cTnT or cTnI for screening asymptomatic patients for the presence of acute coronary syndromes. The likelihood of detecting silent ischemia is extremely low, and cannot justify the costs of screening programs. Additionally, there is also no evidence that cardiac marker analysis of blood following stress testing can indicate the presence of coronary artery disease.

Strength/consensus of recommendation: Class III (for use of cardiac markers for screening).

Session III. Recommendations for Markers in Clinical Applications Other than AMI and Research

Introduction to Section III

The utilization of cardiac troponins in clinical applications other than in patients with ischemic chest pain presenting to rule in and rule out acute myocardial infarction presents several challenges to both clinicians and laboratorians. First, manufacturers of new assays need to work closely with laboratorians and clinicians in designing appropriate clinical trial studies that will a) define an appropriate population to determine the 97.5 percentile reference point; b) define an appropriate patient population to establish an ROC curve derived cutoff for optimal diagnostic sensitivity and specificity for ruling in and out AMI; c) for risk stratification use, define a cutoff for markers in an appropriate population of unstable angina patients based on the assessment of 30 day outcomes for cardiac events. Second, manufacturers need to work closely with professional organizations, such as the AACC and IFCC, as well as national and international cardiology associations to assist in the standardization of both current and future markers.

Inconclusive data are available regarding the prospective clinical use of cardiac troponins for assessment of reperfusion status, infarct sizing, and detection of perioperative AMI in patients undergoing heart surgery, as well as noncardiac surgery. However, a larger body of evidence is accumulating towards the validation of cardiac troponins as sensitive markers of myocardial damage and risk assessment in patients undergoing interventional procedures (PTCA); with similar ordering patterns for AMI rule out being useful. Further, studies have also demonstrated the importance of analytically sensitive troponin assays (second and third generation assays) for the detection and risk assessment in patients with, e.g., congestive heart failure, myocarditis, and sepsis.

The publication of the NACB standards of laboratory practice for use of cardiac markers in coronary artery diseases will serve as a foundation for clinical groups,

especially cardiology, to redefine the AMI definition established by WHO. The impact of new recommendations for troponins and decisive cutoffs have far reaching consequences regarding epidemiology and large trial criteria for patient entrance into studies. Laboratorians, clinicians, and industry need to partner to strengthen international acceptance of current and future cardiac marker recommendations.

Recommendation 1

Acute revascularization is now standard practice for patients with ST-segment-elevation AMI. The objectives for thrombolytic therapy and/or emergent percutaneous transluminal coronary angioplasty are to recanalize occluded arteries and to reduce mortality. Cardiac markers can be used to assess the success or failure of such therapy. AMI patients who develop patent coronary circulation will release a bolus amount of enzymes and proteins into the circulation ("washout phenomenon") when compared with AMI patients with permanent occlusions (76). The accepted standard measurement of reperfusion status is coronary angiography. Blood flow is assessed according to a scale determined by the Thrombolysis in Myocardial Infarction (TIMI) investigators (43). TIMI grades 0-2 indicate various stages of occluded blood flow, whereas TIMI grade 3 indicates reperfusion. The time interval of collection of samples is important for the proper interpretation of results. Methods to predict reperfusion, such as chest pain and ECG resolution, reperfusion arrhythmias, and other criteria, have been shown to be unreliable (77).

Table 3 summarizes the results of some of the several reperfusion studies that have used cardiac markers to assess reperfusion success. When reperfusion was successful, it was produced in the majority of cases within 90 min after the initiation of therapy (78-85). Sampling blood at 60 after the initiation of therapy may be helpful in the early determination of successful reperfusion, but cases of late recanalization could be missed. Some investigators have suggested a 120-min sample (86). Although this time

interval is also acceptable, it could delay any subsequent management decision. Other investigators have used the time to peak marker concentration as the discriminating factor. This is not recommended because it requires more blood sampling and could produce further delays in interpreting results. This is particularly true for patients who have permanent occlusions.

Table 3. Summary of studies on biochemical markers for determination of reperfusion success following intravenous thrombolytic therapy

No. Patients ^a	Marker	Sen/Spec	Aniography Time interval ^b	Reference
7/35	myoglobin	85/100	2 h	78
17/46	CK-MB	85/71	1 1/2 h	79
	myoglobin	94/88		
	cTnT	80/65		
8/17	CK-MB	65/100	1 1/2 h	80
	myoglobin	76/100		
	cTnI	82/100		
12/12	CK-MB	83/100	1 h	81
	cTnT	83/100		
17/32	CK-MB	100/100	1 h	82
	myoglobin	100/100		
52/45	CK-MB	57/54	1 1/2 h	83
	myoglobin	84/40		
	MM isoforms	53/65		
	cTnT	64/54		
8/19	CK-MB	100/61	1 h	84
	myoglobin	100/94		
	cTnI	100/67		
61/146	CK-MB+myo	83/78	1 1/2 h	85

a non-reperfused group/reperfused group.

B Time interval between initiation of therapy and collection of blood.

Recommendation: For assessment of reperfusion status following thrombolytic therapy, at least two blood samples are collected and marker concentration compared:

time=0 defined as just before initiation of therapy, and time=1 as 90 minutes after the start. From these values, the determination of the (a) slope value ($\text{marker}_{t=90} - \text{marker}_{t=0} / 90 \text{ minutes}$); (b) absolute value of $\text{marker}_{t=90}$ minutes; or (c) the ratio of $\text{marker}_{t=90} / \text{marker}_{t=0}$ can be used as the discriminating factors between successful and unsuccessful reperfusion. However, monitoring with biochemical marker strategies has not been successful in distinguishing between TIMI grade 3 from TIMI grade 2 flow patients, rendering the utility of these measurements clinically problematic for determining complete reperfusion.

Strength/consensus of recommendation: Class II.

Recommendation 2

Cardiac markers have also been used to detect the presence of perioperative AMI in patients undergoing surgical procedures (87). The use of nonspecific cardiac markers such as CK, CK-MB, myoglobin, and lactate dehydrogenase have limited usefulness because they are released from noncardiac tissues as a consequence of the procedure itself (88).

The performance of cardiac troponins for the detection of perioperative AMI has been shown to be superior to other cardiac markers such as CK-MB (89,90). However, a protocol for the frequency of blood collection and interpretation of results will require more clinical studies before specific recommendations can be made as to the appropriate decision limit for perioperative AMI. These studies should answer several questions. Can the existing AMI decision limits be used? If the surgical procedure involves the heart, e.g., coronary artery bypass graft, some injury to the myocardium itself is expected. Should a higher AMI decision limit be used in open heart surgeries? It has been shown, for example, that a cTnT concentration of 0.6 ug/L (sixfold higher than

the recommended 97.5% upper reference limit cutoff) had a positive predictive value for an adverse outcome of 87.5%, with a negative predictive value of 98% (91). More studies in which cutoff concentrations are optimized to outcomes are needed.

Recommendation: Cardiac troponin T or I should be used for detection of perioperative AMI in patients undergoing non-cardiac surgical procedures. The same AMI decision limit should be used.

Strength/consensus of recommendation: Class I.

Recommendation 3

Cardiac markers have been used in other monitoring roles, such as myocardial infarct sizing. Infarct sizing involves serial collection of cardiac markers and integrating the area under the curve of a plot of enzyme activity or protein concentration vs time. Such calculations produce an estimate of the quantity of infarcted tissue that correlates to anatomic estimates of infarct size made at autopsy (92). For cardiac markers that exhibit the washout phenomenon, infarct-sizing estimates are inaccurate when reperfusion of occluded coronary arteries is successful (93). Other markers that are not sensitive to reperfusion status, such as myosin light chains (94), may provide more accurate infarct-sizing estimates. However, commercial assays are not readily available for myosin light chains.

Assessment of infarct sizing, however, may be useful as a research tool in clinical trials of new drugs (e.g., intravenous thrombolytic therapy, thrombin inhibitors, and glycoprotein IIb/IIIa inhibitors) or procedures (e.g., angioplasty) designed to limit the extent of myocardial injury, or in studies involving injury that occurs when an occluded artery is suddenly reperfused (95).

Recommendation: Cardiac markers should not be routinely used for infarct sizing because the existing markers are inaccurate in the presence of spontaneous, pharmacologic, or surgical reperfusion.

Strength/consensus of recommendation: Class III (for use of markers in infarct sizing).

Recommendation 4

New markers will continue to be developed and examined for patients with acute coronary syndromes. When a marker such as cardiac troponin demonstrates major advantages over existing markers, there is an urgency of manufacturers to develop and market commercial assays. In the specific cases of CK-MB mass and cTnI assays, there were no cooperative attempts to develop reference materials or to standardize results.

The NACB Committee acknowledges that the exclusive release of new markers may be in the manufacturer's best interests in terms of profitability, and therefore, they may be reluctant to share ideas and needs with their colleagues. Nevertheless, the implementation of new tests is more easily integrated into the laboratory when these markers are available on a wide spectrum of analyzers, and it is in the best interests of the medical community and the in vitro diagnostic industry that assays correlate to one another.

Recommendation: Early in the process, manufacturers should seek assistance and provide support to professional organizations such as the AACC or IFCC to develop committees for the standardization of new analytes. These organizations will determine the need for analyte standardization based on the potential clinical importance of the marker and gather the necessary scientific expertise for the formation of a standardization committee.

Strength/consensus of recommendation: Class I.

Discussion

The IFCC has established the Committee on Standardization of Markers of Cardiac Damage to coordinate the ongoing worldwide activities in this area. This Committee will be working with national clinical chemistry societies, such as the AACC cTnI Standardization Subcommittee, and the German Society for Clinical Chemistry, in their efforts to standardize cTnI and myoglobin, respectively. Although standardization for all cardiac markers is important, it is not urgent for cTnT as this assay is only available from one manufacturer at this time.

Recommendation 5

Utilization of a new test requires the establishment of a reference interval. This is achieved by measuring the concentration of the marker in a cohort of apparently healthy subjects (96). For cardiac markers, a separate "decision limit" is used to differentiate between AMI and non-AMI diagnoses. The decision limit is typically higher than the upper reference limit. Establishment of these limits is essential for the proper interpretation of results.

For cardiac markers, only the upper limit of the reference interval is useful because there is no significance for results that are below the lower reference limit. The limit is defined as the upper 2.5 percentile (one-tail test) of results from a healthy population (96). This statistical approach is commonly used to assign reference interval concentrations (97). For nonspecific markers such as CK, CK-MB, or myoglobin, the reference interval is not ideal, as a higher cutoff concentration is established for clinical decisions. For a specific marker such as cardiac troponin, the upper reference limit is appropriate to establish the presence of cardiac injury (see Session II, "Recommendations 1 and 2").

The AMI cutoff concentration is determined by ROC analysis of results from marker concentrations collected within the established diagnostic time window on a population of consecutive chest pain patients presenting for AMI rule-out. The patients must be diagnosed as having an AMI independent of the experimental cardiac marker being tested, by accepted and rigorously applied criteria (e.g., WHO). However, as part of the AMI diagnosis criteria, one cannot avoid use of accepted cardiac markers (such as CK-MB) that are in routine use at the facility. Recommendations for the standardization of ROC curves have been published (98). These published guidelines suggest that decision thresholds be printed on the ROC curve, the determination of the area under the ROC curve (including standard error and the confidence interval) and calculation of P (or z) when two or more markers are compared on the same ROC plot. Decision limits provided by reagent manufacturers that are not rigorously determined according to the above recommendation should be considered as guidelines and should not substitute for ROC analysis.

Recommendation: Reference ranges are established for each marker on a population of normal healthy individuals using the 97.5 percentile (one-tail) of results. Separate cutoff concentrations for results indicative of AMI are also necessary for all cardiac markers. Standardized receiver operating characteristic (ROC) curves should be used to establish AMI decision limits, using carefully selected and diagnosed patient populations.

Strength/consensus of recommendation: Class I.

Discussion

There was substantial discussion as to how the first troponin cutoff concentration for the detection of myocardial injury should be established. Ideally, this cutoff should be determined empirically with a retrospective analysis of patients with acute coronary syndromes in which the clinical outcomes of these patients are assessed after 4-6 weeks. Using logistic analysis, the value that produces the highest odds ratio for predicting short-term outcomes would be selected as the cutoff concentration. Because such a study is impractical for most hospital laboratories, the upper 2.5 percentile recommendation was made. Other reviewers felt that any detectable troponin indicates cardiac injury, and therefore, the detection limit should be used as the lower cutoff. This might have been acceptable for insensitive assays in which all healthy subjects are below the detection limit. However, improved cardiac troponin assays are being developed that are more sensitive than previous versions, and these assays enable detection of baseline concentrations of cardiac troponin in healthy subjects. Residual troponin concentrations in these subjects represent normal apoptotic turnover of myocardial tissue and not true ischemic myocardial damage (99). Setting the cutoff at the upper 2.5% of the reference population will be directly applicable when more sensitive become available.

Recommendation 6

Much of the focus for new markers has been on the discovery and evaluation of markers that can detect the initial pathophysiologic events of acute coronary syndromes, such as inflammation, thrombus formation, platelet aggregation, and reversible ischemia. Some of the markers examined for these processes include C-reactive protein (100), amyloid protein A (101), thrombus precursor protein (102), p-selectin (103-104), and glycogen phosphorylase isoenzyme BB (105). Other markers that may be used in place of or to improve the specificity of myoglobin include heart fatty acid-binding protein (106) and carbonic anhydrase III isoenzyme (107). Table 4 summarizes the biochemical characterization of these markers.

For research studies involving these new markers, the time of admission is not useful when the results are compared with conventional markers such as myoglobin, CK-MB, and cardiac troponin because the interval between the onset of clinical symptoms and ED admission is variable from institution to institution (108).

Table 4. Summary of early biochemical markers for acute coronary syndromes a

Marker	Biochemical function	size, kDa	Clinical utility
markers of inflammation			
C-reactive protein	acute phase reactant	~120	non-specific
amyloid protein A	acute phase reactant	2.5 (monos) 220-235 (polys)	markers of inflammation
coagulation factors and proteins			
soluble fibrin monomers	soluble protein precursor	??	early detection of
thrombus precursor protein form.	insoluble fibrin	??	thrombus
platelet function			
soluble P-selectin aggregation	platelet activation	140	platelet
ischemic marker			
glycogen phosphorylase BB	enzyme of glycogenolysis	~200	reversible injury
biochemical markers			
carbonic anhydrase III	converts HCO ₃ ⁻ to H ₂ CO ₃	28	skeletal muscle protein (used w/ myo)
fatty acid binding protein	cytosolic fatty acid carrier	15	non-specific early AMI marker

a Modified from Clin Lab News 1996;22:wall poster. Used with permission from the American Association for Clinical Chemistry.

Historical

Recommendation: For research studies involving the kinetics of release and appearance of new biochemical markers, the time course of release and appearance in blood must be defined relative to the onset of clinical symptoms.

Strength/consensus of recommendation: Class I.

The diagnostic accuracy of these new markers may be compromised if the diagnosis of AMI for study patients is based on standard enzyme markers that themselves have sensitivity and/or specificity limitations (e.g., total CK and CK-MB). Therefore, AMI diagnosis should be defined by WHO criteria, but with the substitution of "unequivocal serial changes of cTnT or cTnI" as the principal biochemical marker, in place of the current WHO criteria of "unequivocal serial enzyme changes."

Strength/consensus of recommendation: Class II.

Historical

Session IV. Recommendations for Assay Platforms and Markers of Acute Myocardial Infarction

Introduction to Section IV

The biochemical events that occur following the total occlusion of a coronary artery were summarized by Hearse in 1979, and are still accurate today (109). Figure 7A lists an approximate chronology of these events and the markers that are associated with each step. The initial events occur within the few seconds or minutes after total coronary artery occlusion and are associated with reversible changes. This is characterized by the lack of oxygen delivery, and a reduced production of energy stores (ATP molecules), as the myocyte shifts from aerobic to anaerobic glycolysis and increased glycogenolysis. Enzymes that participate in the breakdown of glycogen such as the phosphorylases are putatively released during this time. In order to conserve energy, there is impairment or failure of the ATP-dependent ion membrane pumps resulting in the release of intracellular electrolytes such as potassium and phosphate (Figure 7A). Concomitant to energy deficits is the inability of the heart to remove waste products. This leads to accumulation and release of metabolites such as lactate and adenosine. Low molecular weight proteins may be able to pass through reversibly injured but repairable membranes.

If the affected artery becomes patent during the early time intervals either spontaneously or by pharmacologic (thrombolytic therapy) or surgical (angioplasty or bypass) means, the jeopardized myocytes can fully recover. Prolonged or permanent occlusion, however, lead to the onset of irreversible damage. The hallmark of irreversible damage is disruption of cellular membranes and release of macromolecules such as enzymes and large molecular weight proteins. The release of mitochondrial proteins in particular, are indicative of cell death and tissue necrosis. Once the marker is released from the myocyte, they must pass through the interstitial space before they can appear in the general circulation. As shown in Figure 7B, ions and low molecular

weight metabolites readily pass through the interstitial space directly into the vascular space. Their appearance into blood (Figure 7C) is rapid. Unfortunately, these ions and metabolites are not specific to myocardial injury, and are not indicative of irreversible damage. Cardiac enzymes and proteins have the advantage of organ specificity, and essentially are only released during irreversible damage. However, they cannot directly pass to the vasculature, and must travel through slow lymphatic drainage. Therefore there is a delay before they appear in blood. The size of the protein and its distribution within the cell dictates the appearance rate. Small intracellular proteins (e.g., myoglobin and fatty acid binding protein) appear first, while large proteins (e.g., CK and LDH) and those that are part of the contractile apparatus (e.g., troponin) have a delayed appearance. Strategies for development of early AMI markers should be focused on proteins that are specific to the heart. In addition, proteins with low molecular weight will appear in blood sooner than large proteins and enzymes.

Historical

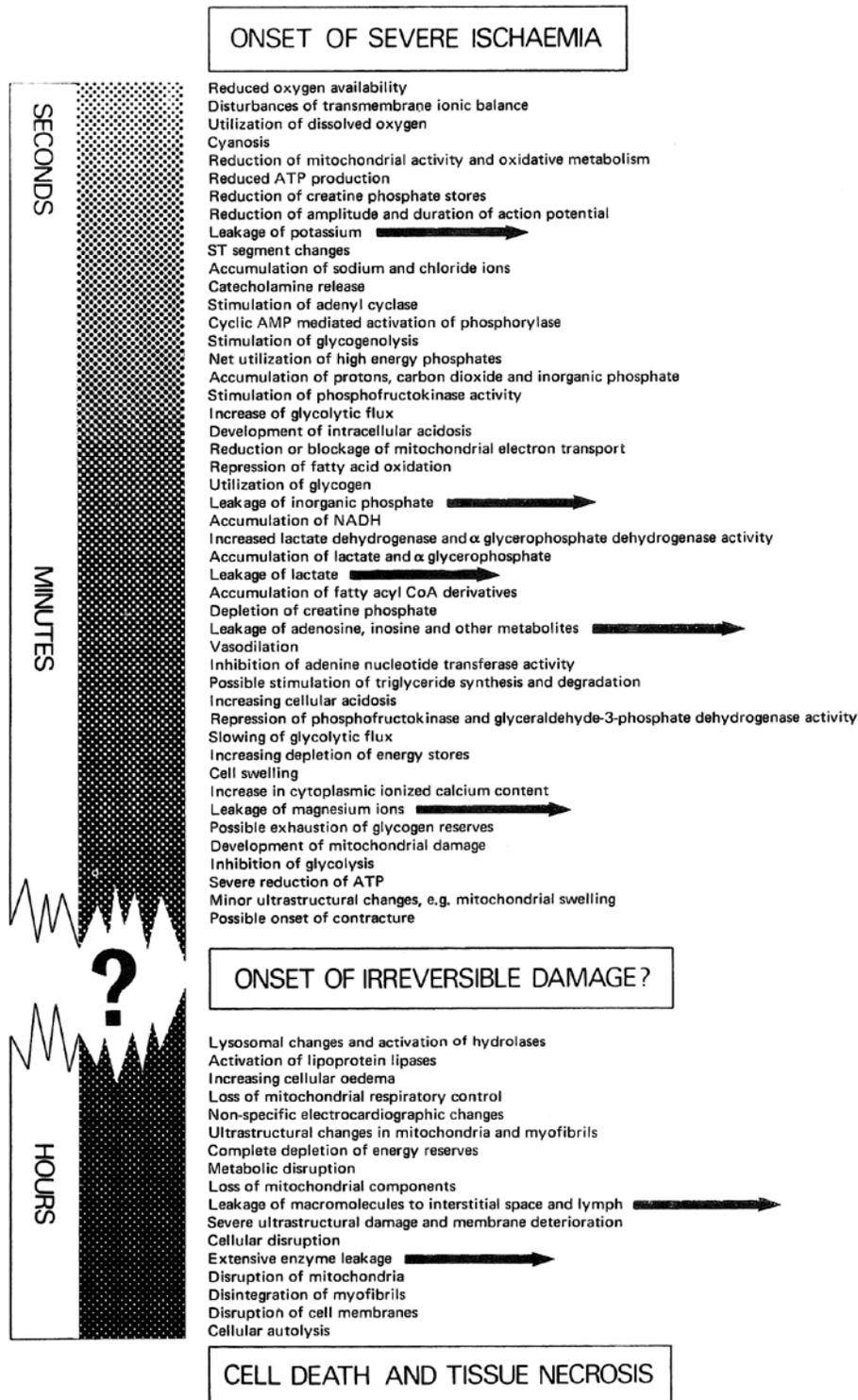


Fig. 7A

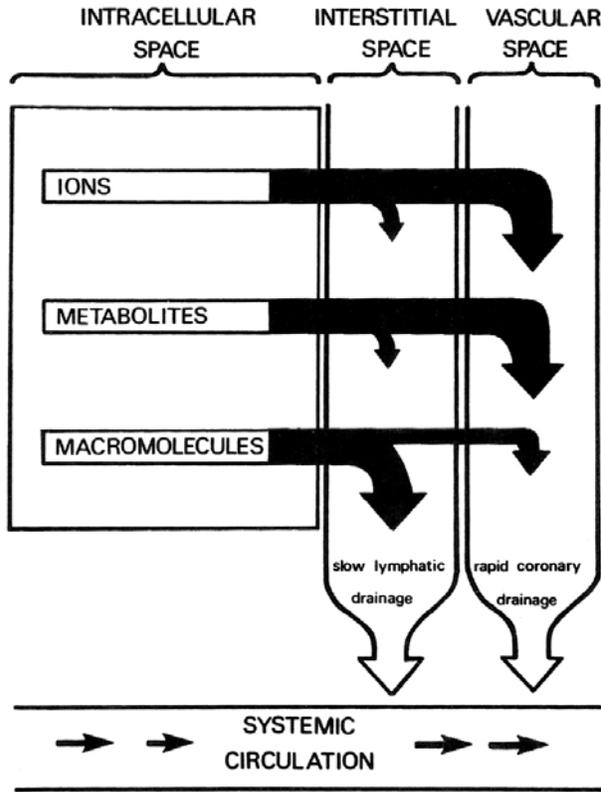


Fig 7B

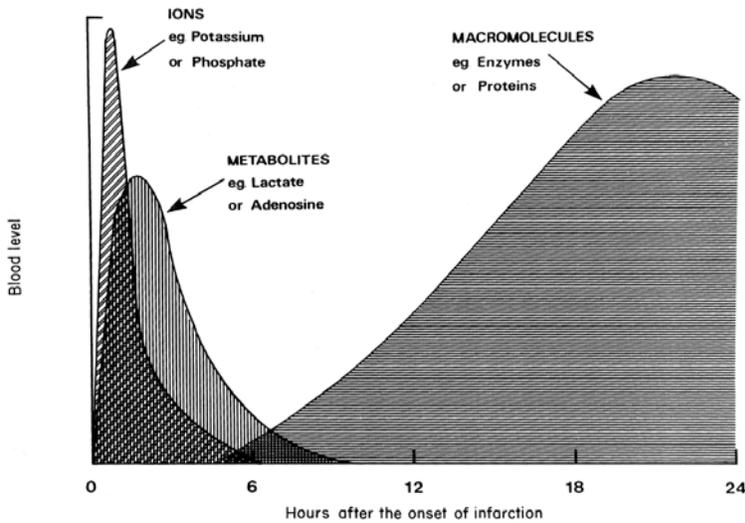


Fig 7C

Fig. 7.

A. List of biochemical events occurring after total occlusion of a coronary artery.

B. Possible routes for release of markers from tissue to blood.

C. Release pattern of marker vs. time after onset of infarction.

Used with permission from Hearse DJ. Cellular damage during myocardial ischaemia: metabolic changes leading to enzyme leakage.

In: Hearse DJ, de Leiris, eds., *Enzymes in cardiology. Diagnosis and research*. Chichester: Wiley, 1979:4-14.

Recommendation 1

CK-MB has long been considered the biochemical standard for the laboratory diagnosis of AMI (110). The development, characterization, and clinical interpretation of cTnT and cTnI seriously challenge the role of CK-MB. cTnT and cTnI appear in the blood at or near the same time as CK-MB, but remain abnormal for 4-10 days (Fig. 3, peak C).

The use of CK-MB should be phased out over the ensuing years as more cTnT and cTnI assays become available and the cost for such assays becomes competitive with CK-MB mass assays (111). If a hospital is already using cTnT or cTnI, the NACB Committee felt that the measurement of lactate dehydrogenase isoenzymes and β -hydroxybutyric dehydrogenase should be discontinued immediately (23,112). No recommendation is being made as to the discontinuance of assays for total CK. This marker is inexpensive and readily available in clinical laboratories, and it can be very useful for the detection of skeletal muscle injury or disease (113).

Recommendation: Cardiac troponin (T or I) is the new standard for diagnosis of myocardial infarction and detection of myocardial cell damage, replacing CK-MB.

Strength/consensus of recommendation: Class II.

There is no longer a role for lactate dehydrogenase and its isoenzymes for diagnosis of cardiac diseases.

Strength/consensus of recommendation: Class I.

Discussion

There was considerable discussion as to whether cardiac troponins can now replace total CK and/or CK-MB. As summarized in Table 5, there are several ongoing analytical issues that have slowed laboratories and clinicians towards a more rapid conversion toward cardiac troponin. For cTnT, the first-generation assay had a problem with nonspecific binding of skeletal muscle troponin (corrected with the subsequent generation of assays). For cTnI, a major issue is the lack of standardization. Results from different manufacturers produce cTnI values that differ by a factor of 20 or more (114). Studies have shown that while the predominate form of cTnI in blood of patients after AMI is the binary complex of cTnI-C, there are smaller amounts of the ternary complex of cTnI-T-C and free cTnI (115). In a study conducted by the AACC cTnI Standardization Subcommittee, biases in results for cTnI among commercially available cTnI assays were caused by the lack of standardization to a single reference material, and the lack of standardization of antibodies used in the cTnI assay. (114). On a molar basis, some assays had equal responses to all forms, some were more reactive to the cTnI complex than free forms, while others had a differential response between binary and ternary complexes (Fig. 8). Epitope mapping studies have shown that the specificity of cTnI antibodies is directly dependent on the specific peptides selected from the cTnI amino acid sequence (116). Antibody selection will also determine the ability of an assay to recognize proteolytic degradation products of cTnI (117). Thus after AMI, the apparent clearance rate of cTnI from blood will vary between assays. Given the complexity of cTnI pathophysiology in blood, harmonization of assay results across commercial platforms have proven to be extremely difficult.

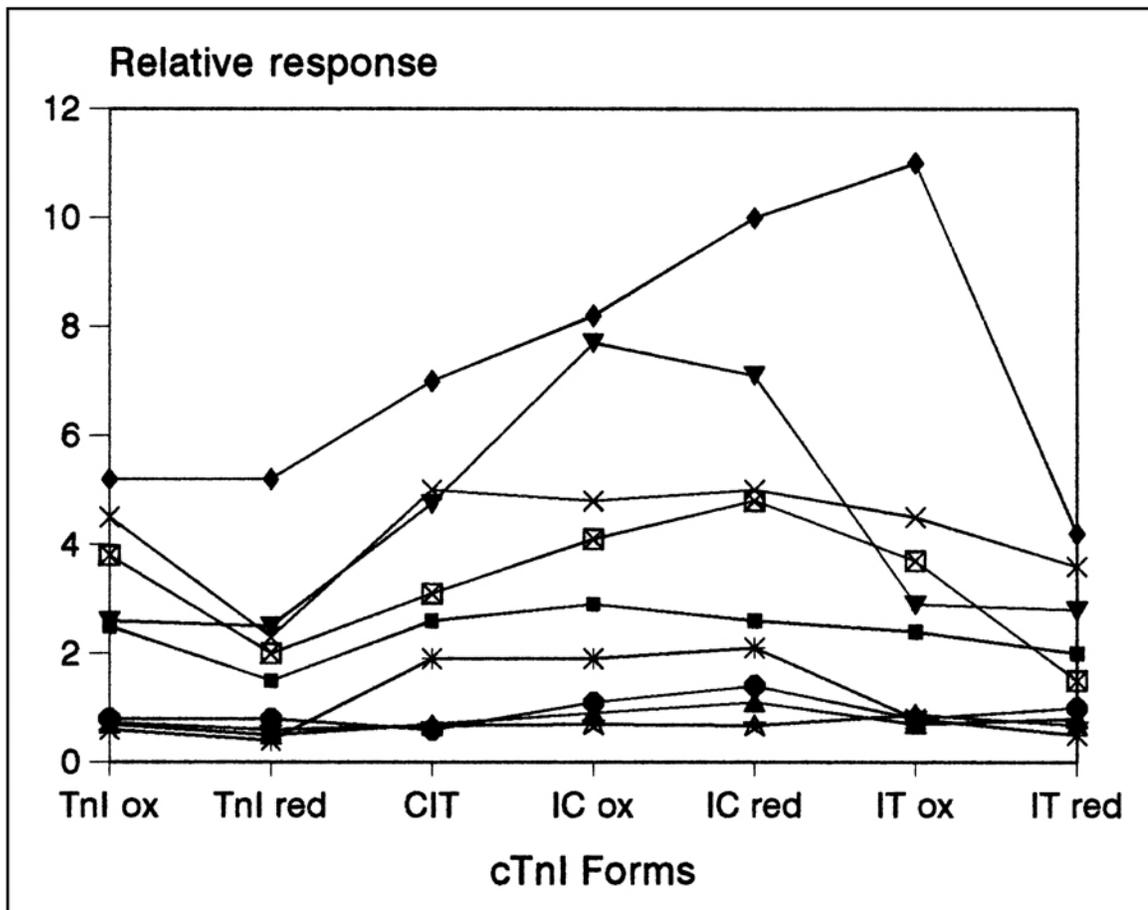


Fig. 8. Relative response for troponin assays to prepared samples of cTnI. Used with permission from the American Association for Clinical Chemistry.

Table 5. Continuing analytical issues for implementation of cardiac troponin as an accepted standard for myocardial injury.

- lack of assay standardization for cTnI
- lack of standardization between laboratory-based and point-of-care testing platforms
- lack of good analytical correlation (e.g., $r > 0.950$) among commercial cTnI assays for clinical specimens
- variability in imprecision for all cardiac troponin assays
- variability in acceptable blood collection tubes
- appropriate cutoff concentrations not documented
- potential for false positive results due to presence of fibrin and human anti-mouse antibodies.

Within-run and total imprecision also are not uniform between commercial assays (118). In many assays for cardiac troponin, the presence of fibrin clots and heterophile antibodies can produce false-positive results (119). These problems have prompted

manufacturers of troponin assays to produce new generation kits to improve assay sensitivity and specificity.

Cardiologists have also expressed concerns about totally replacing CK-MB. Although quantitative calculations using the area under the CK-MB vs time curve are seldom made, many physicians use peak CK-MB to get a qualitative impression as to the size of a myocardial infarction. Others have questioned whether serial troponin measurements can be used for reinfarction (because of the prolonged release pattern) and suggest a continuing role for CK-MB for this purpose. Still others feel that there has not been enough peer-reviewed publications on various cTnI troponin assays or day-to-day experience by practicing cardiologists to warrant a change at this time. The NACB Committee felt that over the ensuing years, most of these issues will be resolved. Therefore, despite the existence of these limitations, hospitals should begin considering the replacement of CK-MB.

An important issue that must be resolved at each institution is reimbursement for these tests. Recently, the Health Care Finance Administration announced that "it is not necessary to use troponin in addition to creatine kinase (CPT codes 82550-82554) (which includes the MB isoenzyme) in the management of patients with myocardial infarctions", suggesting that reimbursement will not be given when both tests are ordered (120). Private insurance companies may also limit reimbursements for cardiac markers (e.g., Blue Cross/Blue Shield of Michigan does not reimburse for cardiac troponin). Although these Guidelines recommend the use of troponin as the new standard for myocardial injury, the NACB Committee recognizes that it is unrealistic for a hospital or medical center to completely change over to cardiac troponin without a "transition period," during which both CK-MB and cardiac troponin assays are offered. The length of the transition period could be 2-3 months, depending on the acceptance and understanding of the use cardiac troponin results by the medical staff and the degree of continuing education available. After the trial period, the data should be

reviewed and a decision made as to whether to a) continue the trial period, b) keep CK-MB, c) replace it with one of the cardiac troponins, or d) make routine use of both CK-MB and cardiac troponin.

During the presentations, the NACB Committee took a poll as to whether a recommendation can be made now to retire CK-MB. The majority felt that CK-MB still had a role. However, when the conference participants were asked about the future (5 years) use for CK-MB, essentially all felt that CK-MB would eventually be abandoned. The NACB Committee has retained this recommendation because the NACB believes that it should take a leadership role in recommending future clinical laboratory practices. The publication of the recommendation as written may provide documentation and assist laboratory directors and administrators to make changes in testing policies sooner. If laboratories are to retain CK-MB, the NACB Committee recommends the use of mass assays, which have been shown to be superior to activity-based assays (such as immunoinhibition or electrophoresis) (36,121). The calculation of the percent relative index $[\text{CK-MB (in ug/L)}/\text{total CK (in U/L)} \times 100]$ may assist in the differentiation between myocardial and skeletal muscle causes of increased total CK (122,123). Other investigators have concluded that the relative index unacceptably degrades the sensitivity of CK-MB and should be abandoned (124,125).

Recommendation 2

AMI patients with ST-segment elevations on the ECG can be effectively treated with thrombolytic therapy, particularly if therapy is initiated within 12 h after the onset of chest pain. Delays in implementation will reduce the success of this treatment. As such, the National Heart Attack Alert Program has made a recommendation to physicians to treat all AMI patients within 60 min of their arrival in the ED (126) (Figure 9A). Results for serum cardiac markers are not needed in making this therapeutic decision. However, rapid testing and reporting of cardiac marker concentrations may produce other benefits

for cardiac patients. Two outcome studies have shown that testing cardiac markers on a continuous random-access basis decreased the length of stay and overall laboratory costs compared with testing on a batched basis (127,128). It is presumed that providing stat testing will lead to more time-efficient decisions for triage and discharge.

The factors that affect TATs include the delay in the delivery of the sample to the laboratory, the preanalytical steps necessary to prepare the sample, the analysis time itself, and the effort it takes to deliver results to the ordering physician. The NACB Committee understands that the time taken for the delivery of samples to the laboratory is not always under the control of the laboratory. Nevertheless, laboratory personnel should work closely with hospital administrators and nursing staffs to minimize delays. TATs can be improved with the implementation of pneumatic tubes that deliver samples directly and rapidly to the central laboratory. The use of satellite laboratories is another mechanism to reduce delivery and, therefore, reporting turnaround times. Fig. 9B summarizes the steps necessary for reporting a laboratory result for cardiac markers.

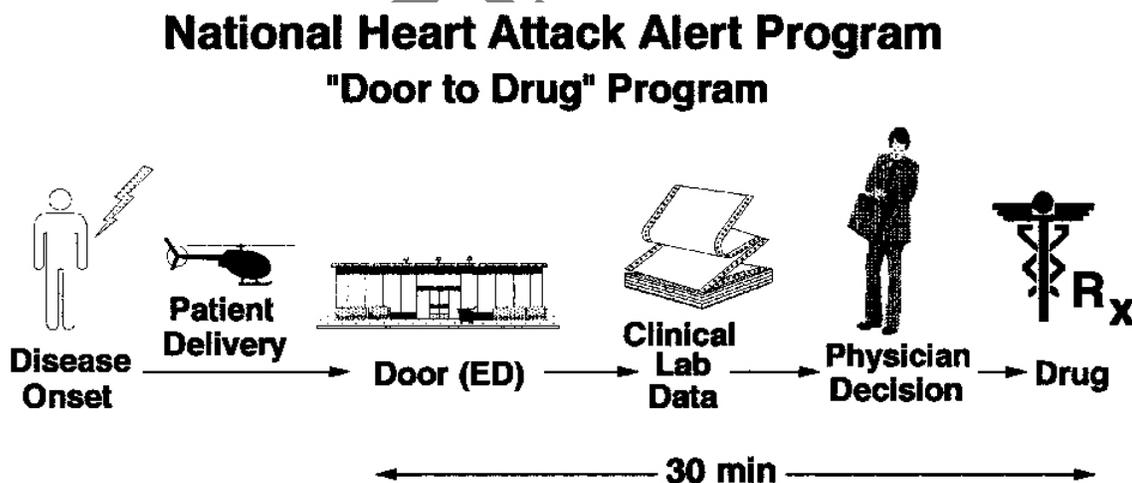


Fig. 9. A. National Heart Attack Alert Program, 60 minutes to Treatment Working Group "Door to Drug" program.

National Academy of Clinical Biochemistry "Arm to Report" Program

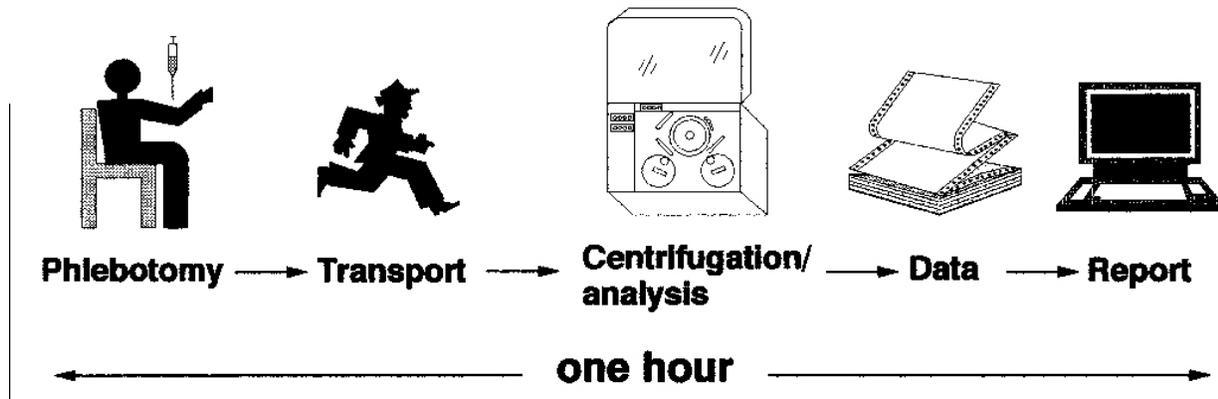


Fig. 9. B. NACB "Arm to Report" recommendation for a 1-hour turnaround time for collection, transportation, analysis, and delivery of results for acute cardiac marker testing.

Recommendation: The laboratory should perform stat cardiac marker testing on a continuous random-access basis, with a target turnaround time (TAT) of 1 h or less. The TAT is defined as the time from blood collection to the reporting of results.

Strength/consensus of recommendation: Class II.

Discussion.

There was considerable discussion on the issue of TAT. There was some support for further reducing TATs. When questioned during the plenary lecture, Dr. Eugene Braunwald responded that 40 min was a target for ED TAT. One reviewer stated that new technologies for sample delivery, bar-coding, and rapid centrifugation will enable laboratories to consistently meet this goal and that the NACB should begin to set very high standards. Decreasing TATs would invariably be received positively by the ED staff if they themselves were not responsible for the testing. On the other hand, other individuals felt that although the technology for rapid TATs exists, many hospitals have limitations in human resources. Thus, if a sample sent from the ED for cardiac markers is accompanied by a request for a complete blood count, blood gases,

electrolyte profile, gram stains, and other tests, the bench technologist must prioritize which test to perform first. When a choice is presented to the ED staff as to which stat analytes should be tested first, a cardiac marker panel might not have the highest priority. Because of the lack of consensus, the NACB Committee has retained the recommendation of a 1-h TAT objective. It is unlikely that a laboratory will be able to consistently (>90%) deliver stat cardiac marker results in 30-40 min, using laboratory-based serum or plasma assays. Results of stat cardiac marker testing are not used to determine the need for thrombolytic therapy. Moreover, rule-out of AMI from the ED does require results of serial sampling, which further diminishes the need for a very rapid TAT on any single sample.

Recommendation 3

Some laboratories do not have automated immunoassay analyzers, rapid tube delivery systems, or staffing to deliver results within 1 h on a continuous basis.

Qualitative as well as quantitative POC testing devices are now available for myoglobin, CK-MB, cTnT, and cTnI (129-132). These assays make use of anticoagulated whole blood, and have analysis times of 20 min. Eliminating the need to deliver samples to the central laboratory and centrifugation enables TATs of 30-40 min. In a recent randomized study, results obtained with POC testing were compared with results obtained in a central laboratory for consecutive admissions to a coronary care unit (133). The POC testing group was associated with a shorter assay TAT (5 min vs 69 min, $p < 0.05$) and coronary care unit length of stay (1.94 vs 2.51 days) compared with testing performed in the central laboratory; because of the small number of subjects, the difference in coronary care unit length of stay did not reach statistical significance. Recently, multipanel quantitative POC testing devices have been approved by the Food and Drug Administration for combinations of myoglobin, CK-MB, and cTnI. Quantitative assays may ultimately be more useful than qualitative POC devices. However, because of the newness of quantitative POC assays, there have

been no studies to compare the effectiveness of qualitative vs quantitative POC testing in the ED. Therefore, the NACB Committee was unable to formulate a recommendation at this time. In some qualitative and quantitative POC testing devices, the total number of analytes measured is fixed. Despite this, the NACB Committee endorses the use of only two: an early (myoglobin or CK-MB mass) and a definitive (cardiac troponin T or I).

Although outcome studies have shown that expedited testing and reporting of results for cardiac markers reduces hospital length of stay and laboratory costs for cardiac patients (127,128), there are no outcome studies to validate the specific need for a 1-h TAT. It is clear, however, that early treatment of Q-wave AMI patients with thrombolytic therapy is important for success in terms of reducing mortality and increasing the rate of coronary artery patency. With the development of new therapeutic strategies for unstable angina and non-Q-wave AMI, the NACB Committee anticipates that early detection of any myocardial injury will also be beneficial in the management of these patients. For those patients who are ruled out for acute coronary syndromes, it is expected that fast TATs for laboratory data will lead to faster patient discharges and a reduction in overall hospital costs. The NACB Committee encourages prospective outcome studies to examine the putative advantage of reporting TATs within 1 h.

Recommendations: Institutions that cannot consistently deliver cardiac marker turnaround times of ~1 h should implement POC testing devices. The cardiac troponin cutoff concentration should be set at the 97.5% upper reference limits so that the devices can detect the first presence of true myocardial injury.

Strength/consensus of recommendation: Class I.

Recommendation 4

POC devices are designed for testing to be performed at or near the bedside by the primary caregivers. However, the responsibility for this testing must reside with the laboratory. The success of POC testing programs will depend on cooperation and the acknowledgment of the laboratory's responsibility by hospital administrators, nursing staffs, and the appropriate units within the hospital (e.g., the ED).

When the laboratory staff recognizes a situation of noncompliance, they should have the authority to direct the corrections, and, if necessary, remove POC testing devices and suspend testing from the area of the hospital where the testing was conducted until the deficiencies have been satisfactorily corrected.

Recommendation: Among other tasks, laboratory personnel must be involved in the selection of devices, the training of individuals to perform the analysis, the maintenance of POC equipment, the verification of the proficiency of operators on a regular basis, and the compliance of documentation with requirements by regulatory agencies such as the Health Care Finance Administration and Clinical Laboratory Improvement Act of 1988. In meeting these requirements, a quality-assurance and quality-control program must be instituted and fully documented on a regular basis.

Strength/consensus of recommendation: Class I.

Recommendation 5

Assays for cardiac markers for early diagnosis, rule-out, triaging of patients from the ED, or for determination of successful reperfusion require markers that have a short assay TAT. Irrespective of how or where the testing is performed (i.e., laboratory-based or POC testing), assays must meet minimum precision requirements. Imprecise assays at or near cutoff concentrations will adversely affect the clinical performance of the test.

Recommendation: Assays for cardiac markers should have an imprecision (CV) $\leq 10\%$ at the AMI decision limits and an assay TAT of <30 min. Before launch, assays must be characterized with respect to potentially interfering substances [e.g., other related proteins, human anti-mouse antibodies (134,135), and other interferences].

Strength/consensus of recommendation: Class II.

Discussion

The NACB Committee understands the importance of establishing objective analytical goals for assays for new cardiac markers. This will assist manufacturers in the construction of new assays. The total precision required for a particular assay is dependent on the biological variation of the analyte. The biologic variation has been established at 5.6% for myoglobin (136) and 9.3% for CK-MB (137). The biologic variation for cardiac troponin has not been established. As such, this recommendation for total precision was arbitrarily set at 10% without a prior scientific basis.

Recommendation 6

Most patients with cardiac diseases are heparinized while hospitalized. When serum is collected from these patients, full clot retraction from tubes without preservatives can take 10-15 min or more. Clots can continue to form even after the sample has been centrifuged and the serum placed onto immunoassay analyzers. When this occurs, fibrinous material can interfere with the assays as well as during

sampling to block probes. For automated immunoassay analysis, the use of plasma will eliminate the extra time needed for clotting, thereby reducing the overall preanalytical TATs. Manufacturers should target their assays for use in plasma as well as provide for safety procedures that will detect clots in samples. Results for serum and plasma are not interchangeable for all assays and markers, particularly for cTnI. Therefore, for cardiac troponin, NACB recommends that laboratories do not intermix different types of blood collection tubes at the same facility.

Although whole blood testing is not yet an option for most automated immunoassay analyzers, it is available for POC testing. The use of whole blood can reduce assay and reporting TATs. Currently, the assay TATs for myoglobin, CK-MB, and troponin are 10-20 min. For some samples, dilutions will be necessary to report quantitative results that are outside the limits of the reportable range. Electronic transmission is essential for the efficient reporting of results.

Recommendation: Plasma or anticoagulated whole blood are the specimens of choice for the stat analysis of cardiac markers.

Strength/consensus of recommendation: Class I.

Discussion.

In the original draft of the Guidelines, the recommendation stated that heparinized plasma is the specimen of choice for troponin measurements. However, some reviewers, particularly those in Europe, suggested that the Guidelines be expanded to include all forms of plasma collection tubes (such as EDTA or citrated collection tubes). Laboratories that choose to use these collection types must proceed with caution. With EDTA tubes, troponin released as a ternary (cTnT-I-C) or binary (cTnI-C) complex will degrade to free subunits because ionized calcium is needed to maintain this complex and is removed by chelation of the metal ions (138). Troponin

assays that do not exhibit an equimolar response between complexed and free subunits will produce significant biases between serum and EDTA plasma (114). Heparin does not disrupt complexes; therefore, no change in results between serum and plasmas are expected. The laboratory must follow the recommendations for acceptable specimen types listed in manufacturers' package inserts and should use a reference interval specific to the specimen type.

General Discussion

One reviewer expressed concern that if these guidelines are enacted, laboratories that choose not to enact one or more of the recommendations may be open to liability if a cardiac patient suffers an unfavorable outcome and a lawsuit is filed. This is an important issue for all clinical practice guidelines committees and expert panels. The Committee believes that these guidelines provide well accepted goals and objectives for laboratories in the use and application of cardiac markers. They do not set absolute standards of practice, rather, they encourage proper use and establishment of future goals.

The objective of the NACB Committee was not to make recommendations as to how cardiac markers are to be used in conjunction with other diagnostic modalities (e.g., electrocardiography, echocardiography, and nuclear imaging ventriculography) or how results should be used to select specific therapies. Organizations such as the National Heart Attack Alert Program Committee and the Agency for Health Care Policy Research have been developed to address such issues. We hope that the clinical organizations, especially cardiology, will use these guidelines to move forward towards updating the WHO criteria for ruling in and ruling out AMI.

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Historical

Table 1. Common cause of chest pain^a

Cardiac	Pulmonary	Others
<i>Ischemic syndromes</i>	Bronchitis	<i>Vascular</i>
Stable angina	Bronchospasm	Aortic dissection
Unstable angina	Empyema	Pulmonary embolism
Variant angina	Pleural effusion	Pulmonary hypertension
AMI	Pleuritis	<i>Gastrointestinal</i>
<i>Valvular disease</i>	Pneumonia	Esophageal spasm
Mitral valve prolapse	Pneumothorax	Gastroesophageal reflux
Aortic stenosis	Pulmonary edema	Mallory-Weiss tear
Subaortic stenosis	Aortic dissection	Esophagitis/gastritis
<i>Cardiomyopathy</i>	Pulmonary embolism	Gastric/duodenal ulcer
<i>Pericarditis</i>	Pulmonary hypertension	Biliary colic
		<i>Musculoskeletal</i>
		Costochondritis
		Muscle strain/spasm
		Cervical radiculopathy
		<i>Neurologic: Herpes Zoster</i>

^aTaken from Green GB, Green SF. Markers of myocardial injury in the evaluation of the emergency department patient with chest pain. In: Wu et al. ed., Cardiac Markers, Totowa NJ: Humana Press, 1998, p. 77.

Table 2. Rate of inappropriate discharge from the ED for patients with AMI

Study	Year	Percentage
Pozen et al. (5)	1984	7%
Tierney et al. (6)	1986	13%
Lee et al. (7)	1987	4%
Rouan et al. (8)	1987	10%
McCarthy et al. (9)	1993	2%
Puleo et al. (10)	1994	5%
Graff et al. (11)	1997	4.5%

Historical

Table 3. Summary of studies on biochemical markers for determination of reperfusion success following intravenous thrombolytic therapy

No. Patients ^a	Marker	Sen/Spec	Aniography Time interval ^b	Reference
7/35	myoglobin	85/100	2 h	78
17/46	CK-MB	85/71	1 1/2 h	79
	myoglobin	94/88		
	cTnT	80/65		
8/17	CK-MB	65/100	1 1/2 h	80
	myoglobin	76/100		
	cTnI	82/100		
12/12	CK-MB	83/100	1 h	81
	cTnT	83/100		
17/32	CK-MB	100/100	1 h	82
	myoglobin	100/100		
52/45	CK-MB	57/54	1 1/2 h	83
	myoglobin	84/40		
	MM isoforms	53/65		
	cTnT	64/54		
8/19	CK-MB	100/61	1 h	84
	myoglobin	100/94		
	cTnI	100/67		
61/146	CK-MB+myo	83/78	1 1/2 h	85

^anon-reperused group/reperused group. ^bTime interval between initiation of therapy and collection of blood.

Table 4. Summary of early biochemical markers for acute coronary syndromes^a

Marker	Biochemical function	size, kDa	Clinical utility
<u>markers of inflammation</u>			
C-reactive protein	acute phase reactant	~120	non-specific
amyloid protein A	acute phase reactant	12.5 (monos) 220-235 (polys)	markers of inflammation
<u>coagulation factors and proteins</u>			
soluble fibrin monomers	soluble protein precursor	??	early detection of
thrombus precursor protein	insoluble fibrin	??	thrombus form.
<u>platelet function</u>			
soluble P-selectin	platelet activation	140	platelet aggregation
<u>ischemic marker</u>			
glycogen phosphorylase BB	enzyme of glycogenolysis	~200	reversible injury
<u>biochemical markers</u>			
carbonic anhydrase III	converts HCO_3^- to H_2CO_3	28	skeletal muscle protein (used w/ myo)
fatty acid binding protein	cytosolic fatty acid carrier	15	non-specific early AMI marker

^aModified from Clin Lab News 1996;22:wall poster. Used with permission from the American Association for Clinical Chemistry.

Table 5. Continuing analytical issues for implementation of cardiac troponin as an accepted standard for myocardial injury.

lack of assay standardization for cTnI

lack of standardization between laboratory-based and point-of-care testing platforms

lack of good analytical correlation (e.g., $r > 0.950$) among commercial cTnI assays for clinical specimens

variability in imprecision for all cardiac troponin assays

variability in acceptable blood collection tubes

appropriate cutoff concentrations not documented

potential for false positive results due to presence of fibrin and human anti-mouse antibodies.

Historical

Figure captions

- Fig. 1. Demographics and outcomes of patients who present to emergency departments in the U.S. with chest pain.
- Fig. 2. Time line for chest pain evaluation centers. From Perfecting MI Ruleout. Best Practices for emergency evaluation of chest pain. Cardiology Preeminence Roundtable, Washington, DC, 1994, used with permission.
- Fig. 3. Plot of the appearance of cardiac markers in blood vs time after onset of symptoms. Peak A, early release of myoglobin or CK-MB isoforms after AMI; peak B, cardiac troponin after AMI; peak C, CK-MB after AMI; peak D, cardiac troponin after unstable angina. Data are plotted on a relative scale, where 1.0 is set at the AMI cutoff concentration.
- Fig. 4. Pathophysiology of acute coronary syndromes. A. Cross-section of coronary artery showing the presence of a lipid-filled plaque with a thin fibrous cap. B. Rupture occurring at the shoulder region of the plaque, which is an area of vulnerability due to high circulatory shear stress. C. Exposure of plaque core elements propagates thrombus formation. D. Totally occlusive thrombus causing AMI. Reprint from Clinical Laboratory News, Jun 1998, page 12-14, with permission from the American Association for Clinical Chemistry.
- Fig. 5. Summary of pathophysiologic events in acute coronary syndromes. Reprint from Clinical Laboratory News, Jun 1996, poster insert, with permission from the American Association for Clinical Chemistry.
- Fig. 6. Cutoff concentration for use of a non-specific marker such as CK-MB have traditionally been set to differentiate between patients with unstable angina and AMI. Use of a biochemical marker that is highly specific for cardiac injury enables the selection of two cutoff concentrations: differentiation between unstable angina vs. AMI, and stable angina vs. unstable angina. Used with permission from Wu AHB, Clin Chim Acta 1998;272:11-21.
- Fig. 7. A. List of biochemical events occurring after total occlusion of a coronary artery. B. Possible routes for release of markers from tissue to blood. C. Release pattern of marker vs. time after onset of infarction. Used with permission from Hearse DJ. Cellular damage

during myocardial ischaemia: metabolic changes leading to enzyme leakage. In: Hearse DJ, de Leiris, eds., Enzymes in cardiology. Diagnosis and research. Chichester: Wiley, 1979:4-14.

Fig. 8. Relative response for troponin assays to prepared samples of cTnl.

Fig. 9. A. National Heart Attack Alert Program, 60 minutes to Treatment Working Group "Door to Drug" program. B. NACB "Arm to Report" recommendation for a 1-hour turnaround time for collection, transportation, analysis, and delivery of results for acute cardiac marker testing.

Historical

Appendix I.

The National Academy of Clinical Biochemistry acknowledges the following individuals who have reviewed drafts of this document and have sent written commentary to committee members or have expressed their views during the Edutrak sessions. In some cases, names were obtained from transcripts of the presentations. We apologize if names or affiliations are incorrectly listed or if we inadvertently omitted anybody.

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Other names were not given or could not be recovered from the tapes of the AACC Edutrak discussion sessions.

Appendix II

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