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 surger, 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 referfusion 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. Patientsa	Marker	Sen/Spec	Aniography Time intervalb	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 ($marker_{t=90}$-$marker_{t=0}$/90 minutes); (b) absolute value of $marker_{t=90}$ minutes; or (c) the ratio of $marker_{t=90}/marker_{t=0}$ 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	platelet activation	140	platelet
aggregation			
ischemic marker			
glycogen phosphorylase BB	enzyme of glycogenolysis	~200	reversible injury
biochemical markers			
carbonic anhydrase III	converts HCO3 - to H2CO3	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.

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.