

IFCC Committee on Standardization of Markers of Cardiac Damage: Premises and Project Presentation

ABSTRACT

The field of biochemical markers of cardiac damage is in a dynamic state, with new applications continually appearing and new assays and markers being developed. These significant and rapid advancements in the development of new biochemical assays have led, however, to several analytic and interpretative problems. In this situation, it is essential that a uniform and rigorous outlook be maintained to ensure optimal test utilization.

For these reasons, the IFCC Scientific Division recently agreed to establish a Committee on "Standardization of Markers of Cardiac Damage" (C-SMCD), inviting members from the already established American and European groups to become members of this Committee. In this presentation, the premises, the issues, and the proposed plan of action of C-SMCD are presented and discussed.

Background

Acute myocardial infarction (AMI) is one of the most common diseases in Western society and is increasing alarmingly in developing countries. Accurate and early diagnosis is important in minimizing cellular damage and, consequently, in obtaining a successful outcome for the patient, especially since the advent of thrombolytic treatment.¹

Historically, the laboratory assessment of cardiac damage has been important in supporting the clinical diagnosis of patients. Twenty years ago, the World Health Organization (WHO) defined the diagnosis of AMI as a triad,² two of which must be present for diagnosis:

1. Typical history of severe and prolonged chest pain;
2. Unequivocal electrocardiographic changes, with ST-segment elevation and the development of abnormal Q wave;
3. Serial enzyme changes, with initial rise and subsequent fall of catalytic concentrations.

Creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) have been first measured as markers of cardiac necrosis, and then subsequently, relatively more specific markers such as CK-MB catalytic activity and LDH isoenzyme 1 have been introduced. Practically, these markers have been used to help establish the diagnosis of AMI and to rule out myocardial necrosis in patients who present with a history compatible with an acute ischemic syndrome.³ However, frequently these traditional markers are also elevated in other noncardiac conditions.⁴ In addition, their sensitivity is low during the early hours after onset of symptoms and patient presentation, and when there is only a minor degree of cardiac damage.⁵

Because of inadequate sensitivity and specificity with the current enzymatic standard for myocardial injury, new biochemical markers have been developed and investigated.⁶ From a clinical point of view, the ideal biochemical marker that detects myocardial injury requires certain properties. This marker should: (a) be present in the myocardium in high concentrations and absent in nonmyocardial tissues for high cardiac specificity; (b) be released rapidly into the blood after myocardial injury so as to achieve optimal sensitivity in the early phase after onset of damage; (c) remain abnormal for several days; and (d) be assayed with a rapid turnaround time. So far, there is

probably no single marker that meets all of these criteria.

Myoglobin is not cardiac-specific. The major false-positive results arise from patients with renal failure or skeletal muscle diseases.⁷ However, myoglobin rises at least 1 hour earlier than any other commercially available marker and can be effective to rule out AMI.^{8,9} Is this useful in practice? Probably, this depends on the use to which the emergency room physician puts this information, which in turn depends very much on local factors. Other markers that appear early in the serum after onset of myocardial damage, such as CK-MB isoforms, heart fatty acid-binding protein, and glycogen phosphorylase isoenzyme BB show promise and are under investigation. Their role at the present time, however, is not well established.¹⁰

Recent advances in analytic techniques have increased the diagnostic values of CK-MB, enabling earlier and more sensitive results.¹¹ The CK-MB mass immunoassays, which utilize the monoclonal anti-CK-MB in conjunction with anti-M or anti-B antibodies, are able to measure accurately small changes during the early hours after AMI with a diagnostic sensitivity of 50% at 3 hours and in excess of 80% at 6 hours after infarction.¹¹ However, CK-MB mass retains two limitations in diagnosing AMI. First, the marker is not specific to cardiac injury, with increases also occurring during massive musculoskeletal injury. Indeed, skeletal muscles contain small but significant amounts (1-3%) of CK-MB.¹² This can cause diagnostic confusion in patients after surgery or trauma. Furthermore, the early release of CK-MB limits its value for the late diagnosis of myocardial infarction. Because of this narrow time window,

FIGURE 1

	Cardiac troponins cutoff limits, µg/l.	
	Healthy subjects	Minor myocardial damage WHO AMI
Boehringer cTnT	0.04	0.20
Dade cTnI	0.40	1.50
Boehringer cTnI	0.50	2.50
Sanofi cTnI	0.03	0.15

AMI: acute myocardial infarction.

Fig. 1: Suggested cutoff limits for commercially available troponin assays.

Table 1 - Biochemical markers of cardiac damage: Present issues.

- ▶ Analytic problems
 - à Test standardization
 - à Test imprecision
 - à Pre-analytic variability
- ▶ Turnaround time (Point-of-care testing)
- ▶ Economic analysis (Costs in relation to expected benefits)
- ▶ Practice guidelines (Diagnostic "Gold Standards")
- ▶ Decision thresholds
- ▶ Prognostic implications

there is, however, a definite place for using CK-MB mass for the diagnosis of re-infarction.

Cardiac troponin I (cTnI) and troponin T (cTnT) are regarded as the most specific of currently available biochemical markers for myocardial damage.¹³ Furthermore, of the new markers for myocardial necrosis, troponins have the widest temporal windows: 5 to 14 days for cTnT and 4 to 10 days for cTnI, respectively. In this period, the increase in troponins is 5- to 10-fold greater than that of CK-MB, the reference standard. Due to their cardiac specificity and their very low concentrations in serum of normal individuals, the cardiac troponins have also a greater sensitivity for minor degrees of myocardial injury. Increase in troponins has been well documented with chest pain syndromes without increase in the activities of traditional enzymes and isoenzymes, and a new diagnostic category—the "minor myocardial injury"—has been created to describe this condition.^{14,15} These increases have also been associated with an increase in short-term adverse cardiac events.¹⁶

The present issues

The significant and rapid advancement in the development of new biochemical assays for myocardial damage have led to some practical problems. Table 1 lists the main issues.

Test standardization. Myoglobin, CK-MB mass, and troponins are all determined by a number of different immunoassays using antibodies directed to different epitopes on the respective antigens. Consequently, different results from different systems and assay generations cloud the interpretation of reported data. This problem is well recognized in the literature.

In a multicenter evaluation of five different commercial methods for the determination of myoglobin carried out by the Working Group of Italian Society of Laboratory Medicine on "Markers of Cardiac Damage," method comparison studies showed high regression coefficients (> 0.98).¹⁷ However, Bland-Altman plots and 95% confidence interval of bias showed that methods by Dade Stratus and Boehringer Hitachi gave results significantly higher than that of Behring BNA, Behring Opus, and Sanofi Access.¹⁷ These biases observed in patients' samples are probably due to a combination of factors, including differences in antibody specificities, matrix effects, and lack of an accepted reference standard for myoglobin. The American Association for Clinical Chemistry (AACC) Committee obtained similar results when it attempted to corre-

late immunoassays for CK-MB mass.¹⁸

Recently, the Cardiac Markers Working Committee of the Laboratory Proficiency Testing Program of Ontario, Canada, analyzed the most recent set of quality control results with regard to the participants using mass assays for CK-MB analyses. They found that the results from Chiron ACS:180 were significantly different from all other results, and there were also significant differences between the two Abbott analyzers, i.e., IMx and AxSYM.¹⁹ It can therefore be appreciated that calibration differences may create a major obstacle to comparing inter-analyzer results.

Similar problems exist also for troponins. In a comparison study between the Opus cTnI method and the Sanofi Pasteur immunoenzymometric cTnI assay, the correlation was good, but the data showed considerable scatter, the results from the Opus method being relatively higher within the midrange of values but equal to the Pasteur assay results at concentrations outside the limit of linearity of the instrument. As a whole, the Opus assay led to approximately 10-fold higher values than the Pasteur assay, the data being hardly comparable.²⁰

In a second study, the Access automated cTnI assay was compared with Opus assay. Again, although the correlation was high, a significant difference in the values obtained with the two methods was demonstrated.²¹ These differences in currently available assays for cTnI determination could be explained by a different specificity of antibodies, purity and type of standard antigens (purified free troponin I or troponin I complexed with troponin C or T), and reaction conditions. Katrukha et al²² suggest that effective and reliable immunologic detection of cTnI is possible only when antibodies used for assays recognize both free troponin I and troponin I complexed with other troponin components present in the bloodstream. McPherson et al²³ added that antibody pairs that recognize oxidized and reduced cTnI are necessary to achieve the most reliable immunoassay of total cTnI. Finally, phosphorylation of cTnI may also affect its immunologic activity.²⁴ A technologic refinement of cTnI assay has also created some confusion.²⁵ The second-generation cTnT assay entirely eliminates crossreactivity from skeletal muscle troponin T, whereas the previous method of testing cTnT had a crossreactivity of about 2%, with the possibility of false-positive results during severe skeletal muscle damage.²⁶

The differences in the upper reference limits and in the clinical decision thresholds using different troponin assays arise directly from this standardization problem.²⁷ For example, using Access analyzer, apparently healthy subjects have concentrations less than 0.03 µg/l.²¹ Respective values for Behring Opus analyzer and Dade Stratus analyzer are less than 0.5 and 0.6 µg/l.^{28,29} Thus, these concentrations differ by more than 10-fold between analyzers.

Test imprecision. At present, at least for myoglobin and CK-MB mass, we know the goals for analytic imprecision, expressed as CV, directly derived from biologic variability studies: 5.6% for myoglobin and 4.2% for CK-MB, respectively.^{30,31} This should provide an objective target for manufacturers of instruments and kits. Using this goal as reference, in the multicenter study previously cited,¹⁷ we have shown that some commercial assays for myoglobin determination do not meet this target of quality at present, and an improvement in the precision of the measurement is required if these assays are to be offered on a routine basis. Regarding imprecision of commercially

available troponin assays, there is at present an inverse correlation between the time required to complete the assay and the imprecision of the methods, the higher CVs being present in the faster analyzers (see Table 2).

Preamalytic variability. Poor attention has been paid so far to possible preanalytic limitations of the cardiac marker determination. The effect of

Table 2 - Precision at diagnostic cutoff level and time required to complete an assay of commercially available troponin assays.

Assay	Time to complete assay (min)	CV
Boehringer cTnT ES300	45	6.8%
Sanofi cTnI Access	20	15.7%
Behring cTnI Opus	20	13.0%
Dade cTnI Stratus	10	13.6%*

Note: All the findings presented are from the author's experience, except for *, which was taken from ref. 32.

Table 3 - Possible interference by anticoagulants in commercially available immunoassays for cardiac troponin.

Assay	Sample type	
	Heparinized plasma	EDTA plasma
Behring Opus	Useful	Not useful
Boehringer Enzymun	Not useful	Useful
Dade Stratus	Useful	Not useful
Sanofi Access	Not useful	Useful

Note: Data from author's experience. Not useful indicates a difference $\geq \pm 6\%$ from values obtained using paired serum samples.

storage time and temperature on apparent marker concentration, the effect of repeated freeze-thaw cycles, and the possible influence of different anticoagulants should be clearly studied. In a study investigating cTnI stability in serum samples using assays with different antibody pairs, recovery of cTnI at 7th day varied with each system and with different samples.³³ On the other hand, we know that different anticoagulants cause interferences with different troponin assays (see Table 3). The same problem was recently shown for myoglobin immunoassays (see Table 4).¹⁷

Turnaround time. Regarding the turnaround time of cardiac marker measurement, at a roundtable organized by the International Society of Clinical Enzymology, held at the University of Cambridge in the summer of 1996, the general opinion of the speakers was that such markers should be available within 20 minutes of ordering the test.³⁴ This is, of course,

of this approach point out the speed and convenience of such testing. Not only can these devices be used in the emergency room or stat laboratory, but also they are available in the patient's home or in the ambulance (Roth et al, unpublished results). An evidence-based appraisal is urgently required of the real need for POC testing in cardiac patients. Clinical and cost-effectiveness studies, and a clarification of the role of central lab in the implementation of POC testing and in its quality control are other issues.

Economic considerations. With increasing economic pressures on the health care system, there is a parallel need to assess cost-effectiveness of diagnostic testing. In other words, we need considerations of cost in relation to expected benefits. This concept is important but has not been applied to the provision of biochemical markers of cardiac damage. Although there is an abundance of literature on cardiac markers, there are only a few articles that evaluate the impact of these tests on outcome.³⁹ With regard to this point, the laboratory has and will continue to play a large part in proper selection and interpretation of available markers, using evidence-based knowledge of test utilization and outcomes.⁴⁰

Practice guidelines, decision thresholds, and prognostic implications. Strictly related to the economic analysis is the issue of practice guidelines. The knowledge of the spectrum of acute coronary syndromes as pathophysiologic continuum suggests that even small elevations of specific markers of myocardial damage, like troponins, should be acknowledged as indicative of significant injury.¹⁵ Even if, from a practical point of view, two cutoff limits are probably needed at present for a convenient use of troponins. Patients with minor myocardial damage as detected by cardiac troponins have significant risk for adverse cardiac events and should be appropriately managed on a prospective basis (see Fig. 1).

Having demonstrated the use of troponins to predict outcome and provide prognosis, the challenge is now to reduce the high risk of subsequent ischemic events in patients with acute coronary syndrome with an elevated troponin test results.⁴¹ On the other hand, the applicability of the cardiac markers as prognostic indicators might also provide a useful tool for the choice of optimal treatment. A study has recently shown that elevation of cTnT identifies a subgroup of patients with unstable coronary syndrome in whom prolonged antithrombotic treatment with a low-molecular-weight heparin can improve the prognosis.⁴² Additional prospec-

Table 4 - Possible interference by anticoagulants in commercially available immunoassays for myoglobin.

Assay	Sample type	
	Heparinized plasma	EDTA plasma
Behring Opus	Useful	Not useful
Boehringer Hitachi	Not useful	Not useful
Dade Stratus	Not useful	Useful
Sanofi Access	Not useful	Not useful

Note: Data from ref. 17. Not useful indicates a difference $\geq \pm 6\%$ from values obtained using paired serum samples.

not always possible: delays of up to two hours or more are not uncommon in clinical practice. The laboratory must, however, report results with the fastest turnaround time possible. The utility of this approach was showed in a study,³⁵ in which the laboratory changed its cardiac markers testing policy from batch testing twice per day using electrophoresis to stat testing of CK-MB mass and myoglobin immunoassays, with a turnaround time of one hr, 24 hours a day. In the subgroup of AMI patients with nondiagnostic electrocardiogram, the patients whose serum were tested for biochemical markers under the batch policy had a longer hospital stay than patients tested under a stat protocol. The same results were seen in the group of patients in whom AMI was ruled out, those tested under a batched protocol had a stay of 4.4 days, as compared to three days for stat testing.

Table 5 - Major tasks of the IFCC Committee on Standardization of Markers of Cardiac Damage (C-SMCD)

- ▶ Coordination of the different activities of various committees from affiliated associations working on cardiac markers with:
 - a review of documents and recommendations;
 - the cooperation with standardization activities.
- ▶ Support the development of the scientific programs for the forthcoming international meetings and congresses.
- ▶ Development of specific additional projects on standardization and clinical use of the markers.

tive outcome studies are, however, needed to document whether antithrombotic agents and/or aggressive revascularization treatments can improve the prognosis of troponin-positive patients.

National and international activities

Currently, several associations have formed committees working on various aspects of markers of cardiac damage. Under the leadership of the German Society of Laboratory Medicine, various societies of German-speaking countries with different medical backgrounds have created a "Committee on Standardization of Immunoassays" in 1994 with the main focus on cardiac markers. Two main goals of the Committee are: the standardization of laboratory parameters and the elaboration of diagnostic strategies. Regarding standardization, the Committee decided to concentrate its activity on myoglobin. The objectives of the program were: (a) the evaluation, characterization, and the selection of appropriate materials that can allow common calibration of immunoassays for measurement of this marker, (b) the assignment of a myoglobin concentration to the selected material using a consensus method, and (c) the achievement of an international approval for the certified reference material. However, some problems have prevented the practical realization of this program so far. The second goal of the German Committee was the elaboration of diagnostic strategies based on laboratory testing of cardiac markers for an effective use of such markers in the diagnosis and management of acute ischemic heart disease, and a position paper has been recently published.⁴³

The AACC has at present two subcommittees working on CK-MB mass and cTnI standardization. The objective of the CK-MB Subcommittee was to identify and characterize a standard material, based on human enzyme, that can be used to improve the accuracy of commercial CK-MB mass assays.⁴⁴ Recently, the Committee concluded that a lyophilized form of recombinant CK-MB is useful as a reference material for standardizing CK-MB mass assays. The overall bias between the CK-MB methods was reduced from 40% to 12% with the use of this reference material.

In 1996, AACC established a subcommittee for standardization of cTnI immunoassays. The subcommittee's primary mission is to develop and characterize a consensus reference material for cTnI to minimize between-method variation, through the selection of appropriate materials and the evaluation of their reproducibility, stability, and analytical recovery performances.

Two years ago, the Italian Society of Laboratory Medicine (SIMeL) in cooperation with the Italian Society of Clinical Biochemistry (SIBioC) created a Working Group on "Markers of Cardiac Damage." This group has performed an evaluation of the performance of various commercially available immunoassays for myoglobin.¹⁷

At the European level, the Standards, Measurements, and Testing Program of the European Commission supports a project on "Certification of the mass concentration of CK-MB in a reference material." The objective of the work is to assign a mass concentration value to the already prepared CK-MB Reference Material 608, with previously certified catalytic activity. Finally, the U.S. National Academy of Clinical Biochemistry proposed "Recommendations for the Use of Cardiac Markers in Coronary Artery Diseases" as part of "1998 Standards of Laboratory Practice" and discussed these guidelines in a forum during the AACC 1998 Meeting in Chicago.

Tasks of the IFCC Committee

As previously discussed, the field of biochemical markers of cardiac damage is in a dynamic state, with new applications continually appearing and new assays and markers being developed. These developments have raised several analytic and interpretative problems. In this situation, it is essential that a uniform and rigorous approach be maintained to ensure optimal test utilization. For these reasons, after some preliminary discussions, the IFCC Scientific Division recently agreed to establish a Committee on "Standardization of Markers of Cardiac

Damage" (C-SMCD), inviting members from the established American and European groups to become members of this Committee, thus stressing collaboration with IFCC.

A plan of action of C-SMCD has been developed taking into account the work already done by the existing National and International groups (see Table 5). The first urgent task is the coordination of the different activities of these groups, with a review of documents and recommendations and the supervision and, possibly, the take-over of standardization activities. In addition, the C-SMCD will support the development of scientific programs for the forthcoming international meetings and congresses. The C-SMCD, via its chairman, is also directly involved in the scientific organization of the forthcoming 7th Bergmeyer Conference "Markers of Cardiac Damage—Current Status and Future Trends," scheduled for the 1st of February 1999. Finally, the C-SMCD will be involved in the development of additional specific projects to promote the rational use of cardiac markers and to identify new areas of research.

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