13. RECOMMENDATIONS ON USE OF BIOCHEMICAL MARKERS IN ACUTE CORONARY SYNDROME: IFCC PROPOSALS

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13.1 Introduction

Proper evaluation of the patient with acute chest pain is a resource-intensive and expensive process. Critical to the effective management of these patients are the early recognition of a cardiac ischaemic event and the proper placement of the patient in the risk spectrum of the acute coronary syndrome. With increasing economic pressures on health care, physicians, health plans, and medical centres are interested in improving the efficiency of care for patients with acute chest pain. This interest recently reinforced the need for a better diagnostic approach to patients with suspected acute coronary syndrome and, consequently, the need for a new standard definition of acute myocardial infarction (AMI) and of risk determination.

For much of the past three decades, acute ischaemic heart disease has been regarded as a binary phenomenon, AMI or non-AMI, using World Health Organization recommendations that included fulfilment of at least two of the three well-known diagnostic criteria: a history of acute, severe, and prolonged chest pain; presence of significant changes in electrocardiogram (ECG); and unequivocal abnormal elevation of traditional enzyme activities in serum. Chest pain is, however, an unreliable indicator: up to 33% of patients with AMI may have no chest pain and are clinically silent on presentation to the hospital. The ECG remains the cornerstone for the early diagnosis of acute ischemia, showing in approximately 60% of patients ST-segment change within seconds of the ischemic insult. However, the ECG can be inconclusive in the remaining 40% of cases, therefore showing a globally low sensitivity. The imperfect sensitivity and specificity of the traditional enzymatic markers for the detection of myocardial injury is also well known.

In this historical context, the risk of misdiagnosis was therefore relatively high. Several studies estimated that 2 to 8% of patients with AMI were inadvertently sent home from emergency departments because of the diagnostic limitations of the ECG and of measurements of classic enzymes. Inappropriate early discharge also resulted in significantly higher morbidity and mortality.

13.2 Approaching a new standard for diagnosis

Considering these pitfalls in the traditional criteria for diagnosis of AMI and the excellent findings of several clinical trials using highly sensitive and specific markers of heart muscle damage that are not themselves enzymes, such as cardiac troponins, the Committee on Standardization of Markers of Cardiac Damage (C-SMCD) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) made a recommendation in 1999 to expand on the enzyme diagnostic criteria for AMI to include cardiac-specific proteins. However, the C-SMCD considered that it was the responsibility of cardiology groups, and not laboratorians, to officially redefine the biochemical criterion for diagnosis of AMI. The consensus document published in 2000 by the European Society of Cardiology and the American College of Cardiology is therefore the appropriate next step, making specific new recommendations on the use of biomarkers for the detection of myocardial necrosis. In particular, the document considers as the best biochemical indicator for detecting myocardial necrosis "a concentration of cardiac troponin exceeding the decision limit on at least one occasion during the first 24 hours after the onset of clinical event". The use of creatine kinase MB (CK-MB), measured by mass assays, is still considered as an acceptable alternative only if cardiac troponin assays are not available,. The redefined criterion used to classify acute coronary syndrome patients presenting with ischaemic symptoms as AMI patients is therefore heavily predicated on an increased cardiac troponin concentration in blood.

Cardiac troponins are correctly regarded as the most cardiac-specific of currently available biochemical markers for the diagnosis of myocardial injury. In particular, cardiac troponin I (cTnI) and cardiac troponin T (cTnT) have been identified. These proteins are associated with specific amino acid sequences encoded by genes different from those encoding skeletal muscle isoforms. The cumulative data indicate that troponins appear in the serum relatively early after AMI onset (4 to 10 hours), peak at 12 to 48 hours, and remain abnormal for 4 to 10 days. These release kinetics can be accounted for by examining the distribution of the proteins within the myocardial cell. The great majority of both cTnI and cTnT is bound to the myofibril (94 to 97%) and a relatively small amount (approximately 3% for cTnI and 6% for cTnT, respectively) is free in the cytoplasm. After a cardiac cell is injured and the free cytoplasmic pool is immedi-

Table 1. Elevation of cardiac troponins in patients without overt ischemic heart disease

- · Acute rheumatic fever
- · Amyloidosis
- · Cardiac trauma (including contusion, ablation, pacing, firing, cardioversion, cardiac surgery)
- · Cardiotoxicity from cancer therapy
- · Chronic renal failure
- · Congestive heart failure
- · Critically ill patients, especially with diabetes
- · Hypertension, including gestational
- · Hypotension, often with arrhythmias
- · Myocarditis
- · Postoperative noncardiac surgery
- · Pulmonary embolism
- · Sepsis

ately released, there is slow continuous release of the proteins bound to myofibrils, resulting in the observed prolonged troponin elevations noted before. It should be remembered that cardiac troponins reflect myocardial damage but do not indicate its mechanism. Thus, an elevated value in the absence of clinical evidence of ischemic heart disease should prompt a search for situations in which various degrees of myocardial injury may be present (Table 1). These deserve increased attention for two important reasons: these injuries are frequent in clinical practice, and a significant relationship often is shown between cardiac troponin values and disease severity.

Cardiac troponins should therefore replace CK-MB testing as the diagnostic "gold standard" for myocardial necrosis. Some cardiologists however express concerns about totally replacing CK-MB. Many physicians use the peak serum concentration of this isoenzyme to qualitatively estimate infarct size. Others have questioned the use of serial troponin measurements for monitoring reinfarction (because of the prolonged release pattern) and suggest a continuing role for CK-MB for this purpose. With regard to the first point, it was recently showed that a single measurement of plasma cTnT concentrations performed at the time corresponding to the slow continuous release after AMI, i.e. ~72 hours after onset, can be used as a convenient and cost-effective, noninvasive estimate of infarct size, revealing a similar reliability as peak CK-MB measurement (requiring however repetitive sampling) or nuclear imaging (too expensive to be routinely used). If the major concern about totally replacing CK-MB with cardiac troponins in hospital institutions is the lack of evidence on the ability of troponins to estimate the infarct size, these findings may thus support the definitive implementation of cardiac troponin testing and the replacement of CK-MB in the laboratory cardiac panel.

It may also be appropriate to monitor the continuing decline of CK-MB daily to show an extension of the infarct. As only ~4% of AMI patients experienced a reinfarction during the stay in Coronary Care Unit, the standard monitoring of this marker to obtain this information could however not be cost-effective. Anyway, if laboratories have to retain CK-MB for this particular use, the recommendation is to use the mass assays, which have been shown to be clearly superior to activity-based assays (such as immunoinhibition or electrophoresis).

13.3 The suggested biochemical strategy

An important point concerns the selection of the most appropriate strategy for the use of new markers and the suggested sample frequency in patients with chest pain and without ECG evidence of AMI at hospital admission. In fact, the excitement of new applications in the use of biomarkers to improve routine patient care can be offset by the anxiety regarding the appropriate selection and utilization of currently available and new assays.

Two strategies have competed in this area: -

- The first relies on the use of a combination of two markers – a rapid rising marker, such as myoglobin, and a marker that takes longer to rise but is more specific, such as cardiac troponin – to enable detection of AMI in patients who present early and late after symptom onset. As demonstrated in a systematic review of literature, myoglobin is currently the marker that most effectively fits the role as an early marker. Myoglobin is detectable in blood as early as 2 to 3 hours after onset. Its concentration appears to peak quickly, reaching the maximum concentration between 6 and 12 hours after the onset of symptoms. It then falls to normal concentrations over the next 24 hours, and is rapidly cleared from the serum by the kidneys. Measurement of myoglobin has the merit of robust scientific evidence, with more than 30 studies recently

published on the use of this protein as an early sensitive marker for excluding AMI. Myoglobin has therefore potential utility as test for excluding early AMI in patients presenting to the emergency department with chest pain. The negative predictive value of this marker for excluding early infarction 4 hours after hospital admission is virtually 100%.

This two-marker strategy is predicated on the assumption that early diagnosis of AMI will change care by providing the ability to discharge patients earlier, thus improving flow within the emergency department, and by facilitating identification of patients who may be candidates for aggressive interventions and, more in general, facilitating the triage of patients who are admitted to various parts of the hospital. Various papers clearly document the high performance of the two-marker approach, showing that the combination of myoglobin and troponin significantly improves the clinical predictive values of standard CK-MB alone. In an experience in the use of the two-marker protocol for diagnosis of chest pain, the percentage of acute coronary syndrome-negative patients discharged in less than one day rose from 28% in the control group using traditional enzymatic approach to 50% in the group evaluated by the two-marker protocol. Patients discharged in less than half a day also rose from 22% in the control group to 37% in the test group. The diagnostic information provided by the two-marker strategy significantly improved the accuracy and timeliness of diagnosis of acute coronary syndrome while reducing length of stay and patient episode cost.

• The second strategy suggests that the urgency is less critical than suggested by the first strategy. The tactic involved is simply to measure cardiac troponin, with the understanding that definitive exclusion or inclusion of AMI will take longer. The logic of this strategy insists that for those hospitals who do not have an area for rapid rule-out of patients with chest pain, and therefore patient triage decisions are not made within the first few hours after hospital admission, the use of an early marker is unnecessary. In this case, only measurement of cardiac troponin is suggested with a sampling frequency of admission, 6 and 12 hours. Once again, if compared with the traditional enzymatic approach, this protocol is markedly effective in altering patient management by enabling early discharge of patients, resulting in significant cost savings and increasing bed availability without compromising patient outcome.

Coming back to the sampling protocol for detection of AMI using the strategy employing early and late markers, the IFCC C-SMCD recommends specimen collection at hospital admission, 4, 8, and 12 hours later (Table 2).

Table 2. Proposed diagnostic strategy and recommended sampling frequency for detection of acute myocardial damage by biochemical markers

Marker	Sample frequency			
	Admission	+4 h	+8 h	+12 h or next morning
Early	X	X	(X)	
Troponin	X	X	X	X

This approach enables the association of the high predictability of myoglobin in excluding AMI within 4 hours after hospital admission and the diagnostic power of a single positive result for troponin that would trigger a diagnosis of myocardial necrosis, without the need for necessary completing the sequence of blood samples at every time point. The question of whether zero time in the protocol should be assigned to the onset of chest pain or presentation to the hospital is debatable. Patients with large infarcts tend to have a clear-cut start to the symptoms and to present early, but normally these are not the patients in whom there is any doubt about the need for hospital admission. In the patients with no ECG changes and possible small myocardial damage, the symptoms may have a stuttering start and undergo a waxing-and-waning time-course that mirrors the waxing-and-waning myocardial ischemia. It is not uncommon for these patients to report multiple episodes of chest pain over the hours and days prior to hospital admission and, in about 15% of them, an inaccurate estimation of the time interval between onset of symptoms and admission has been shown. The suggestion is therefore that, for routine clinical practice, blood collections should be referenced relative to the time of presentation to the hospital: the use of the recommended early and late marker combination will permit infarct timing in any case.

13.4 Selection of decision limits for troponin use

One of the most important problems in the practical use of the cardiac-specific troponins is the right definition of decision limits. The basic question is: "How much necrosis is needed to make the diagnosis of AMI?" In the purest physiologic sense, the answer is that any detectable necrosis is an AMI. Consequently, even small elevations of specific markers of myocardial damage, such as cardiac troponins, should be acknowledged as indicative of significant injury, reflecting the incremental risk associated with increasing concentrations of the marker, consistent with the continuous injury concept of acute coronary syndrome. From a clinical perspective, there is clear evidence that any amount of detectable cardiac troponin release is associated with an increased risk of new adverse cardiac events. Currently available data demonstrate no threshold below which elevations of troponin are harmless and without negative implications for prognosis. The 'Fragmin During Instability in Coronary Artery Disease' (FRISC) study, performed in 1996, already showed the continuous relation between cTnT concentrations and the risk of clinical events. More recently, the FRISC-II study confirmed that optimal risk stratification in patients with acute coronary syndrome can be achieved with use of a cut-off concentration around the detection limit of the cTnT assay (i.e., 0.03 mg/L) instead of the manufacturers' suggested higher cut-off (i.e., 0.10 mg/L). Similar results were originally demonstrated for cTnI in the 'Thrombolysis in Myocardial Infarction' (TIMI)-IIIB trial and, more recently, confirmed in TIMI-11B substudy, where use of the upper reference limit concentrations produced significant odds ratios with the three cTnI assays employed.

On the basis of all these evidences, the cardiologists' consensus document quoted before now defines myocardial necrosis as an increase of cardiac troponin values exceeding the upper limit of the normal healthy population, set at the 99th percentile of value distribution to limit the number of false-positive designations of myocardial injury. Pragmatically,

Table 3. Implication of the analytical imprecision of some troponin assays for the diagnosis of acute myocardial infarction (AMI) ***

Company/Platform	Calculated 99th URL*	Concentration associated with a 10% CV**
Abbott AxSYM	0.30 mg/L	0.84 mg/L (2.8 x URL) (1)
Bayer ACS:180	0.07 mg/L	0.30 mg/L (4.3 x URL) (2, 3)
Bayer ACS:Centaur	0.15 mg/L	1.40 mg/L (9.3 x URL) (4)
Beckman Access 2^ gen.	0.04 mg/L	0.06 mg/L (1.5 x URL) (5)
Byk-Sangtec Liaison	0.036 mg/L	0.054 mg/L (1.5 x URL) (6)
Dade Dimension RxL 2^ gen.	0.07 mg/L	0.15 mg/L (2.1 x URL) (7, 8)
Dade Status CS	0.03 mg/L	0.06 mg/L (2 x URL) (9)
DPC Immulite	0.40 mg/L	1.20 mg/L (3 x URL) (10)
Ortho Vitros	0.10 mg/L	0.35 mg/L (3.5 x URL) (11)
Roche Elecsys 3^ gen.	0.01 mg/L	0.03 mg/L (3 x URL) (12)

URL = upper reference limit; CV = total coefficient of variation; gen. = generation.

* Troponin cut-off for AMI, as suggested by European Society of Cardiology/American College of Cardiology

** Troponin cut-off for AMI, as suggested by IFCC C-SMCD.

*** Literature regarding Table 3 is placed at the end of this chapter .

the use of this approach as a diagnostic criterion for AMI will lead to an increase in the numbers of infarct patients in the acute coronary syndrome population from 15 to 30%. However, the document emphasizes that in applying the proposed new diagnostic criteria to clinical practice, patients should not be labeled simply as "myocardial infarction", but rather as patients with coronary artery disease in whom the extent of myocardial necrosis should be clearly defined as microscopic, small, medium or large and possibly related to the current left ventricular function. On the other hand, increasing diagnostic sensitivity for AMI can have a positive impact on society, resulting in more cases being identified, thereby allowing appropriate secondary prevention and hopefully reducing health care costs in the future. In a recent study, patients who had an AMI diagnosis made solely on the basis of a positive troponin value experienced a 3-fold increase in short-term mortality compared with the normal troponin group.

According to the suggestions of the cardiologists' document, the diagnostic manufacturers must now provide on the package insert sheet of kits the 99th reference limit of the specific troponin assays, based on informations available from peer-reviewed literature and obtained using the IFCC recommendations on the theory of reference values, published in a series of articles during 1987. Lacking between-assay standardization, reference limits need, of course, to be determined separately for each assay and platform, even if available from the same manufacturer. This information should be available along with the

level of analytical imprecision of the assay at this concentration limit. Accurate discrimination between "minor" myocardial injury vs analytical noise requires assays that have high precision at low troponin concentrations. For clinical use, the IFCC C-SMCD recommends for troponin assays a total imprecision, expressed as coefficient of variation (CV), of <10% at the AMI decision limit. A failure to reach this goal could increase the risk of reporting misleading results that will either prompt unnecessary confirmatory testing, as in the case of artifactually abnormal concentrations, or lead to clinical inaction when inappropriately low concentrations are reported for patients. This places a large responsibility on the manufacturers of troponin assays to ensure that their assays have the necessary precision to permit the use of the proposed cut-off, i.e. the 99th percentile limit of the reference population. At present, and from this point of view, not all the troponin assays perform equally well in routine clinical settings, and many commercially available assays cannot indeed meet the 10% CV recommendation at the 99th percentile values. Clinical laboratories should therefore consider more carefully the effect of imprecision on clinical decision making when they implement an assay for troponin determination.

On the other hand, manufacturing industries should carefully consider this critical issue because diagnostic and therapeutic decisions will onwards be based on lower cardiac troponin cut points. From a practical point of view, in the contest of clinical practice, for troponin assays that cannot presently meet the 10% CV at the 99th

percentile value, a predetermined higher concentration that meets this imprecision goal should be used as cut-off for AMI until the goal of a 10% CV can be achieved at the 99th percentile (Table 3). Of course, this could however decrease the overall clinical sensitivity of the assay.

13.5 Conclusion

New biochemical markers are integral to the diagnosis and management of patients in whom acute coronary syndrome is suspected. The role of biochemical testing as a part of a structured decision-making protocol is to provide accurate and timely information that can be used to guide patient management. In this respect, the diagnostic superiority of the new markers of myocardial damage opens fascinating perspectives for the triage and management of patients with acute myocardial ischemia.

Recommended literature:

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