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Acute coronary syndrome biomarkers

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Background

The significance of the contribution of Laboratory Medicine to clinical cardiology has grown in importance over the last years[1]. Highly sensitive and specific cardiac biomarkers have become available, assigning to the Laboratory a pivotal role in the diagnosis and follow-up of patients with cardiac disease[2]. This is witnessed by the recent incorporation of these markers into new international guidelines and in the redefinition of myocardial infarction (MI)[3-5].

While the previously used World Health Organization (WHO) definition required the presence of at least two of three criteria, namely, an appropriate clinical presentation, typical changes at electrocardiogram (ECG), and raised "cardiac" enzymes, essentially total creatine kinase (CK) or its MB isoenzyme activities, the new definition of acute MI, proposed in 2000 by the joint European Society of Cardiology (ESC) and American College of Cardiology (ACC) committee, requires the rise and fall of the biochemical marker of myocardial necrosis together with other criteria, comprising ischemic symptoms, the development of pathologic Q waves at ECG, ischemic ECG changes or a coronary artery intervention (Figure 1)[3]. Thus, according to the WHO definition, an acute MI could be diagnosed without biochemical evidence of myocardial necrosis, while the new ESC/ACC criteria stipulate that the biomarkers be elevated and, subsequently, be shown to fall in the appropriate clinical context.

Quite simultaneously with the ESC/ACC redefinition of MI, other expert committees published companion documents, where, in patients with no ST-segment elevation at ECG but with ischemic symptoms, a positive cardiac troponin result identifies patients who have non-ST-segment elevation MI (NSTEMI) and who could benefit from aggressive medical therapy (Figure 2)[4,5].

Correct implementation of new diagnostic criteria

The new consensus documents have based the new definition of MI on biochemical grounds, a choice that was guided by the advent of new markers of myocardial necrosis, such as cardiac troponins[6]. The superior troponin's clinical value comes from its higher sensitivity to smaller myocardial injury and its virtually total specificity for cardiac damage. However, the

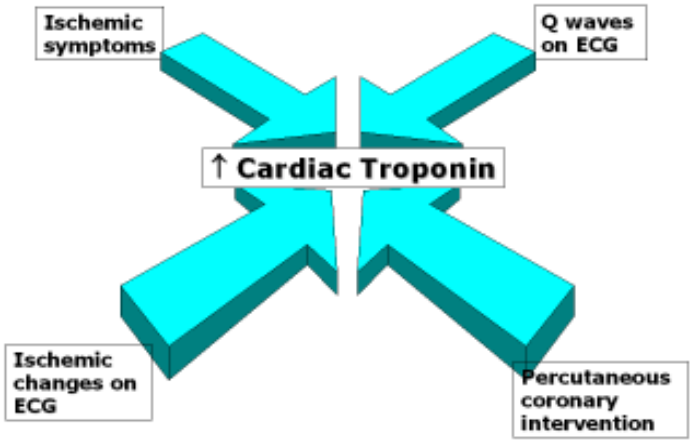

Figure 1. New millennium criteria for acute, evolving or recent myocardial infarction. Source ref. 3. ECG, electrocardiogram.

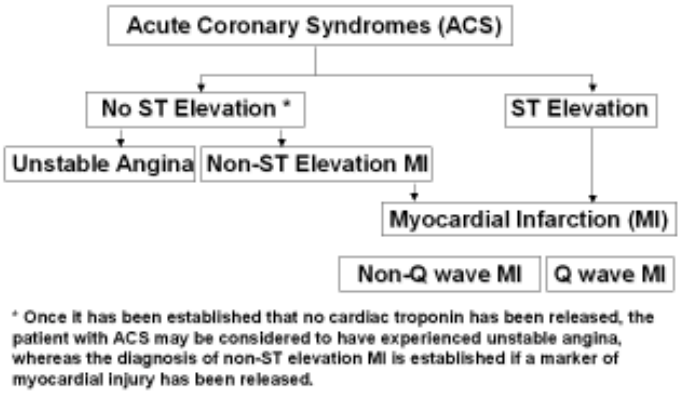

Figure 2. American College of Cardiology/American Heart Association guidelines for management of patients with unstable angina and non-ST-segment elevation myocardial infarction (NSTEMI). Source ref. 4.

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Table I. Cardiac diseases other than acute myocardial infarction causing elevation of cardiac troponins in serum.

- Acute rheumatic fever
- Amyloidosis
- Cardiac trauma (including contusion, ablation, pacing, firing, cardioversion, catheterization, cardiac surgery)
- Cardiotoxicity from cancer therapy
- Congestive heart failure
- Critically ill patients
- Diabetic ketoacidosis
- End-stage renal failure
- Glycogen storage disease type II (Pompe's disease)
- Heart transplantation
- Hemoglobinopathy with transfusion hemosiderosis
- Hypertension, including gestational
- Hypotension, often with arrhythmias
- Hypothyroidism
- Myocarditis/Pericarditis
- Postoperative noncardiac surgery
- Pulmonary embolism
- Sepsis

Table II. Suggested operative cutoffs, compared with the corresponding method-dependent 99th percentile reference limits, for commercially available cardiac troponin assays for the diagnosis of myocardial infarction. Data derived from ref. 15.

Company/Platform	*Suggested cutoff[a], µg/L*	*99th percentile limit[b], µg/L*
Abbott AxSYM	1.22	0.30
Bayer ACS:180	0.37	0.10
Bayer Centaur	0.33	0.10
Bayer Immuno 1	0.34	0.10
Beckman Coulter Access[c]	0.06	0.04
BioMerieux Vidas	0.36	0.10
DiaSorin Liaison	0.06	0.03
Dade Dimension RxL[c]	0.26	0.07
Dade Stratus CS	0.10	0.07
DPC Immulite One	0.32	0.20
Ortho Vitros ECi	0.44	0.10
Roche Elecsys[c, d]	0.04	0.01
Tosoh AIA 21[c]	0.09	0.06

[a] This corresponds to the lowest troponin concentration that can be reliably measured using the corresponding assay with an imprecision (expressed as total CV) ≤10%.
[b] Data obtained from manufacturer's package insert or through personal communications with manufacturers.
[c] These are 2nd or 3rd (Roche) generation assays.
[d] The Roche assay is the only cardiac troponin T assay on the market; all other assays are for cardiac troponin I.

cardiac specificity of cardiac troponin should not be confused with specificity for the mechanism of cardiac injury. In applying the results of cardiac troponin testing to the defining of MI, one should keep in mind that these markers actually reflect myocardial necrosis but do not indicate its mechanism. As clearly reported in the ESC/ACC consensus document, MI and myocardial necrosis are not necessarily synonymous[3]. Thus, an elevated troponin value in the absence of clinical evidence of myocardial ischemia related to coronary atherothrombotic disease should prompt a search for other causes of cardiac

damage, e.g., hypoxia (lack of oxygen), chemical injury, physical (electrical, temperature, radiation) injury, immunologic injury, or infectious agents. Many non-ischemic pathophysiological conditions can cause myocardial necrosis and therefore elevations in cardiac troponin concentrations (Table I). An experience showed that as many as 20% of patients admitted to general medical beds (non-coronary care unit admissions) and with elevated cardiac troponins do not have conventional acute coronary syndrome (ACS)[7]. The occurrence of myocardial damage in clinical contexts other than MI obliges physicians to determine whether such damage occurs in the clinical setting of acute myocardial ischemia, thus leading to the diagnosis of MI, or not. Strictly speaking, even in the "troponin era", the diagnosis of MI remains clinical[8]. Measurement of cardiac troponin provides a valuable diagnostic test for MI only when used together with other clinical information[9]. To satisfy the diagnostic criteria for MI, troponin elevations should be accompanied by objective instrumental evidence that myocardial ischemia is the likely cause of myocardial damage[10]. This should particularly be the case when only one marker measurement is available and its characteristic release kinetics cannot be demonstrated, or when marker changes remain stable over time or are not consistent with the onset of symptoms. Ideally, three measurements of cardiac troponin are suggested, with a sampling frequency of hospital admission, 6 and 12 hours later, to demonstrate changing values[11]. This biochemical strategy can readily show if the temporal variations in the troponin concentrations in serum are consistent with the onset of symptoms and may very often obviate the need for subsequent extensive confirmation testing.

Another important issue in the practical use of cardiac troponins is the appropriate definition of decision limits. From a clinical perspective, there is 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[12-14]. In agreement with the outcome studies, the ESC/ACC consensus document defines myocardial necrosis as an increase of cardiac troponin values which exceeds the upper reference limit (URL) of the healthy population, set at the 99th percentile of the value distribution to limit the number of false-positive designations of myocardial injury[3]. On the basis of current available data, however, it would seem reasonable to expect analytical methods to give an undetectable value or a very low troponin value as "normal". The detection limits and the analytical sensitivities of troponin assays do not *yet al*low the accurate measurement of normal cardiac troponin in healthy subjects and, therefore, the 99th percentile of the reference distribution cannot be calculated with analytical reliability. None of the commercially available troponin assays has shown acceptable analytical imprecision at these low concentration values to obtain accurate discrimination between "minor" myocardial injury and analytical noise[15]. A predetermined higher cardiac troponin concentration that meets the requested goal for desirable imprecision, i.e. a total CV less than 10%, has therefore been proposed as operative cutoff for MI until the assays are improved (Table II)[16]. The use of the actual 10% CV troponin concentration, instead of the lower 99th percentile reference limit, as decision cutoff in the context of clinical practice could slightly decrease the clinical sensitivity of the biochemical criterion used for the MI diagnosis, but should permit physicians to avoid the occasional spurious increase in serum troponin concentrations resulting from analytical noise[17]. It is important to note that, lacking standardization of assays measuring cardiac troponin I (cTnI), the values generated for the same blood sample usually significantly differ between assays, so that clinical thresholds need to be determined separately for each assay and platform. More than 15 different companies presently market assays for cTnI measurements by employing different standard materials and antibodies with different epitope specificities[18]. Consequently, results from one cTnI assay to another can differ by as much as 20-fold and this problem may cloud the interpretations of reported data. Clinicians must be cognizant of intermethod variation and should not generalize decision limits established for one assay to others.

Earlier assessment of ACS

Despite the undoubted ability to detect quantitatively smaller degrees of myocardial necrosis, cardiac troponins are not early markers[19]. These biomarkers need 4 to 12 hours after hospital admission to appear in serum, although they may remain abnormal for several days after symptom onset[20]. Thus, there is still a need for the development of earlier markers that can reliably rule out myocardial damage from the emergency room at patient presentation and, hopefully, detect myocardial ischemia even without the presence of irreversible myocyte injury. Currently, both industry and academia are relentlessly producing an intense research effort to find new serum biomarkers that are released very early during myocardial ischemic injury. Under investigation are two main classes of indicators: markers of early ischemic injury and markers of coronary plaque instability and disruption[21]. Some of these biomarkers have demonstrated promise and need to be more thoroughly evaluated for commercial development for implementation into routine clinical and laboratory practice.

Recent publications have explored the rationale for diagnosing myocardial ischemia in advance (or in the absence) of the occurrence of irreversible damage[22]. As the explicit goal is to maintain microcirculatory flow to prevent even minor infarctions, only a marker that precedes necrosis and permits the prevention of its consequences can meet clinical needs[23]. A marker of cardiac ischemia could also be valuable in distinguishing acute MI from non-ischemic causes of myocardial necrosis that lead to increases in cardiac troponins. The highest expected benefit of an ischemia test would, however, be to rule out ACS in low to moderate pre-test probability conditions with negative necrosis markers and a negative ECG. For this reason, research is ongoing to assess the potential impact of use of newly proposed ischemia markers in an emergency department (ED) population, especially looking at clinical sensitivity and negative predictive value for detecting ACS.

Blood concentrations of free fatty acids unbound to albumin (FFAu) are one of the proposed ischemia tests[24]. During acute myocardial hypoxia, the acute lipid mobilization from adipose tissue can lead to serum concentrations of FFAu in excess of the primary binding sites of albumin in blood. Therefore, FFAu have been evaluated for the early identification

Table III. Synopsis of ischemia-modified albumin (IMA).

1. The circulating protein in blood is modified by exposure to ischemic tissue;
2. The modified protein can be measured with a colorimetric test which detects differences in metal ion (cobalt) binding;
3. Increases in IMA could be observed during ischemia in any vascular bed. Thus, the specificity of the measurement of IMA for myocardial ischemia warrants additional investigation.

of cardiac ischemic injury. Two groups of investigators have preliminarily studied the sensitivity of this marker at patient presentation to the ED and have shown that FFAu elevations may occur well before other more traditional markers of cardiac necrosis, such as CK-MB[25,26]. The sensitivity of FFAu increase at admission was >90% in both studies.

The discovery that albumin, in the serum of patients with myocardial ischemia, exhibited lower metal-binding capacity for cobalt than the albumin in serum of normal subjects was originally made by Bar-Or *et al*. in 2001[27]. Based on these observations, an assay was developed in which the cobalt not sequestered at the N-terminus of albumin is detected using a colorimetric indicator. In sera of normal subjects, more cobalt is sequestered by albumin leaving less cobalt to react with the indicator. Conversely, in sera from patients with ischemia, less cobalt is bound by the ischemia-modified albumin (IMA), leaving more free cobalt to react with indicator. Significant changes in albumin cobalt binding have been documented to occur minutes after transient ischemia induced by balloon angioplasty and to return toward baseline within 12 hours[28]. In a recent study, the sensitivity of IMA at the ED presentation for an ischemic origin of chest pain was 82%, compared with 45% of ECG and 20% of cardiac troponin. All three tests combined identified 95% of patients whose chest pain was attributable to ischemic heart disease[29]. Additional clinical evidence across diverse ED settings is however needed to support these preliminary claims. Furthermore, increases in IMA have been observed during ischemia related to the injury of organs other than myocardium. Thus, the specificity of the measurement of this marker for myocardial ischemia seems to be low (Table III).

A growing understanding of the importance of atherosclerotic plaque rupture in the pathogenesis of coronary events has led to the identification of an expanding array of markers of plaque instability[21]. Experimental studies have demonstrated that phospholipase D enzyme activation and consequent release of choline in blood are related to the major processes of coronary plaque destabilization[30]. Based on these processes, increased blood concentrations of choline have to be anticipated after plaque disruption and myocardial ischemia in patients with ACS. In a prospective study, choline detected troponin-negative patients with high-risk unstable angina with a sensitivity and specificity of 86%, while, of course, traditional markers of necrosis, such as CK-MB and myoglobin, failed to detect high-risk patients[31]. Additional studies are, however, needed to fully investigate the clinical significance of this marker.

Pregnancy-associated plasma protein A (PAPP-A) is known as a high-molecular weight glycoprotein synthesized by the syncytiotrophoblast and is typically measured during pregnancy for Down syndrome screening. It was reported to be an insulin-like growth factor (IGF)-dependent IGF binding protein-4 specific metalloproteinase, thus being a potentially pro-atherosclerotic molecule through its role in increasing concentrations of local bioactive IGF, thereby causing the plaque to proceed to disruption[32]. In pregnancy, PAPP-A circulates in a heterotetrameric complex consisting of two PAPP-A subunits covalently bound with two subunits of the proform of eosinophil major basic protein, its endogenous inhibitor. Conversely, PAPP-A released during atherosclerotic plaque disruption seems to be in a homodimeric active form, uncomplexed with its inhibitor, thus making it difficult to measure PAPP-A as cardiac marker by immunoassays which are designed to detect molecules in pregnancy[33]. Bayes-Genis *et al*.[34] showed the presence of PAPP-A in unstable plaques from patients who died suddenly of cardiac causes and described increased PAPP-A concentrations in the serum of patients with both unstable angina and acute MI. PAPP-A measurement appeared to be valuable for detecting unstable ACS, even in patients without elevations of biomarkers of necrosis, such as cardiac troponins, thus potentially identifying high-risk patients whose unstable clinical situation might otherwise remain undiagnosed. Indeed, the overall correlation of serum PAPP-A with cardiac troponin concentrations appears to be poor, indicating that elevated PAPP-A in ACS cannot be attributed to myocardial necrosis[35].

Troponin testing during percutaneous coronary interventions

One of the major criticisms related to the new ESC/ACC recommendations is the definition of the periprocedural MI. The committee recommended that any detectable rise for biomarkers in the setting of percutaneous coronary intervention (PCI) be considered a MI[3]. It is however hard to accept that the occurrence of micronecrosis, detected by cardiac troponin in about 40% of cases during or after an otherwise successful coronary angioplasty, should be labelled as a MI. Peri- and postprocedural MI is still an unresolved issue and the introduction of cardiac troponins has further focalized interest in this controversy. While it is widely agreed that an increase in serum biomarkers is indicative of myocardial necrosis, the prognostic significance of such changes is still subject of debate. In particular, the amount of increase that is clinically relevant and the marker cutoff that has prognostic significance remain elusive[36].

With regard to the prognostic significance of a rise in CK-MB, prospective studies have suggested a correlation between substantial increases of the marker, i.e. >5 times URL, and the long-term mortality following a PCI[37,38]. Data regarding cardiac troponins are still comparatively scarce. Till now, four large retrospective studies have evaluated the prognostic values of determination of cardiac troponin after PCI (Table IV)[39-42]. Among those, only one showed an adverse prognosis for patients in whom troponin reached a peak 32 times the URL of the employed assay[40]. In another study the increased risk was lost at the mid-term follow-up, and two other studies were unable to show a significant correlation between troponin

Table IV. Major retrospective studies evaluating the prognostic value of cardiac troponin after percutaneous coronary interventions.

Author (year)	*No. of patients*	*Assay (cutoff[a])*	*Follow-up (months)*	*Prognostic value*
Fuchs S *et al.* (2000)[39]	1129	Access[b] (15 x URL)	8	No (but increased risk for in-hospital events)
Nallamothu BK *et al.* (2003)[40]	1157	AxSYM (32 x URL)	12	Yes
Kini AS *et al.* (2004)[41]	2873	AxSYM (20 x URL)	12	No
Natarajan MK *et al.* (2004)[42]	1128	Access[b] (16.7 x URL) or AxSYM (5 x URL)	12	No

[a] Expressed as times x upper reference limit (URL).

[b] 1st generation assay.

elevation after PCI and mortality. Thus, no definitive evidence yet exists on the prognostic value of troponin increase following coronary interventions. Preliminary prospective studies seem to reveal the existence of a cutoff value above which there is a significant correlation between the troponin increase and the mid- and long-term prognoses[43,44]. In contrast to acute spontaneous myocardial necrosis, this threshold seems, however, to be many times the URL. In fact, if a diagnosis of MI always implies the recognition of myocardial damage related to coronary atherothrombotic disease, the severity of the two conditions (spontaneous and iatrogenic) may not be comparable since it is also influenced by other variables including the plaque instability and the residual ventricular function. It is therefore possible that these two populations have differing prognoses even though they have the same magnitude of troponin elevation[45]. Whilst awaiting definitive studies on this topic, in the current clinical practice the post-revascularization iatrogenic damage, shown by isolated cardiac troponin elevation, should be considered as a non-infarction lesion (even though this might seem a contradiction in terms)[10]. In fact, in contrast with spontaneous ischemia, the demonstration of a direct and continuous relationship between the troponin increase after PCI and prognosis remains uncertain. In the meantime, physicians should continue to rely on more conventional CK-MB marker (although measured by sensitive mass immunoassays), using standard post-infarction therapies when concentrations increase above 30 μg/L[9]. It should be recognized that patient outcome and prognosis might be significantly improved by the revascularization procedure despite a small periprocedural myocardial damage. The benefit of revascularizing a stenotic artery should far outweigh the negative impact of a small, especially asymptomatic, biomarker elevation.

Conclusions

In the recent years, sophisticated biochemical markers have become increasingly important in the investigation of ACS and play now an important role in the detection of disease, risk stratification and therapeutic decisions. Nevertheless, someone have underlined that the cardiospecificity and sensitivity of new cardiac biomarkers could be a two edged sword when these markers are used in clinical practice[8]. As the introduction of new biomarkers is a reflection of the scientific progress, their total acceptance is, however, inevitable. The transition from past may be smoother if educational efforts focusing on the conceptual reasoning behind their use and on more controversial aspects of their practical application parallel new biomarker introduction.

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