Cardiac markers of acute coronary syndromes: is there a case for point-of-care testing?

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Abstract

Objective: Major challenges for physicians include selection of effective tests in the time-sensitive identification and management of patients with acute coronary syndromes (ACS). We review whether cardiac marker testing performed at the point-of-care (POC) has an impact on clinical management and guidance of intervention for ACS patients.

Design and Methods: Evidence from recently published studies and meta-analyses supports the efficacy of cardiac markers. Technologies and specifications of all currently available POC tests for monitoring cardiac markers are surveyed. Finally, a series of questions to investigate the utility of cardiac markers, and their measurement by POC tests, for clinical management and guidance of therapy for ACS patients, are addressed.

Results: Cardiac troponins are clearly the best markers for the definitive detection of myocardial infarction. Compelling evidence for the utility of troponins in risk stratification and guidance of intervention for ACS patients has resulted in inclusion of cardiac markers in clinical guidelines. Rapid multi-analyte POC tests, few of which exhibit harmony with central laboratory assays, have facilitated the use of cardiac markers for clinical management and guidance of therapy.

Conclusions: Given the need to minimize vein-to-brain time, it is expected that point-of-care testing of cardiac markers will take a leading role in management of ACS patients. © 2002 The Canadian Society of Clinical Chemists. All rights reserved.

Keywords: Troponin; Creatine kinase; Myoglobin; Myocardial infarction; Cardiac ischemia; Point-of-care testing

1. Introduction

We are in a new era for identifying and treating patients whose coronary artery diseases fall within the spectrum of acute coronary syndromes. In the early 1990s, when it became clear that reducing time-to-treatment of ST-elevation patients with thrombolytic therapy led to better outcomes, the National Heart Attack Alert Program (NHAAP) made the recommendation that physicians treat all patients within 30 min of arrival in the Emergency Department (ED) and began the very successful “4 D’s” initiative [1]. The first “D” stands for door (patient presentation), the second “D” signifies data (obtain electrocardiogram (ECG), blood pressure, etc.), the third “D” is for decision (synthesis of information to determine treatment plan), and the fourth “D” is administration of drug (thrombolytics) to appropriate patients. Treatment of ST-elevation patients is critically important; however this group comprises less than 50% of acute coronary syndrome patients. Over the past decade knowledge has increased regarding non-ST elevation acute coronary syndrome physiology. Parallel advances have been made in therapeutics and development of better biochemical markers, i.e., troponin. These advances have initiated a new era and expanded the second of the “4D’s” (data) to include troponin, and the fourth “D”, (drug) to include administration of GP IIb/IIIa inhibitors and low molecular weight heparin (LMW) heparin to high-risk patients.

Here we review the pathophysiology of acute coronary syndromes, markers of cardiac injury, clinical applications of cardiac markers, Point of Care (POC) testing, current cardiac POC assays, and justification for using POC testing of cardiac markers in the clinical setting.

2. Markers of cardiac injury

Heart disease is the leading cause of mortality in the developed countries, accounting for approximately 500,000
deaths per year in the United States alone [2]. Currently cardiovascular disease affects nearly 70 million Americans, and each year an estimated 8 million patients present acutely to ED with symptoms of ischemic heart disease [3]. About 5 million patients will be admitted to medical centers, of which 1.1 million patients are diagnosed as having myocardial infarction (MI). The remaining three million patients are released from the ED, but of these about 1 to 3% are misdiagnosed and have high risk for adverse outcome [4]. These misdiagnosed patients account for the largest source of malpractice dollars awarded in Emergency Medicine [3,4]. The overall yearly financial burden of heart disease in the US is believed to exceed 10 billion dollars [5].

Biochemical markers of cardiac injury play an essential role in the diagnosis, prognosis, monitoring, and risk stratification of suspected heart attack patients, and have become a central part of therapeutic and interventional guidelines for clinicians. For these reasons, cardiac marker measurements have become a ubiquitous service available at all medical centers having urgent care areas. Although cardiac markers have traditionally been performed exclusively in the central laboratory, over the past 25 yr availability of cardiac marker measurements has evolved from once per-day batches (weekdays only), to several batches per day seven-days a week, and finally to STAT availability. More recently, POC testing for cardiac markers has been driven by the time-critical clinical need for immediate cardiac marker testing, for patient risk stratification, disposition, expedite appropriate treatment and improved outcomes.

3. Pathophysiology of acute coronary syndromes

3.1. Coronary artery atherogenesis

Coronary artery plaques develop from atherogenesis, which begins early in life when lipid-rich deposits containing macrophages and T-lymphocytes exist in the aorta shortly after birth and increase with age [6]. By early adulthood, fatty streaks are present in a large proportion of individuals as demonstrated by autopsy studies of young Korean War casualties [7]. With increasing age, expanding lesions become more numerous and may affect normal laminar blood flow. The lesions contain smooth muscle cells that form a fibrous plaque. In more advanced lesions, the fibrous plaque may become vascularized, and the size of the lipid-rich core increases depending on anatomic location and a number of genetic and life-style risk factors including: age, gender, family history, blood pressure, smoking habit, and blood lipid concentrations [8]. Comorbidities including diabetes mellitus and the metabolic syndrome are also recognized as important risk factors [8]. Further, the inflammatory process is an important component of atherogenesis [9] and infectious disease may also play a role, although the nature of this effect is poorly understood at present.

3.2. Acute coronary syndromes: a continuum of disease

Rupture of unstable coronary plaques and the resulting thrombus formation are the causal pathologies underlying MI [10]. The overview displayed in Fig. 1 indicates that plaques may become unstable and rupture at any age causing “acute coronary syndromes”, a continuum of ischemic disease ranging from unstable angina, associated with reversible myocardial cell injury, to frank MI with large areas of necrosis. Fig. 2 shows that rupture of an atherogenic fibrous plaque in a focal segment of an epicardial coronary artery exposes subendothelial proteins such as collagen and...
von Willebrand factor (vWF) to circulation, leading to adhesion via the platelet’s surface receptors [11–13]. This interaction triggers a change in the platelet’s shape and the enzyme thrombin is generated on the membrane of activated platelets from circulating prothrombin. Thrombin converts fibrinogen to fibrin that will form polymers that make up the framework of a thrombus, trapping mainly platelets in “white clot” (Fig. 2). These clots may also trap red and white blood cells. Because fibrin and vWF are multivalent, they bind to multiple activated platelets leading to their aggregation [14].

Stable angina usually involves stenosis caused by plaques resulting from years of atherosclerotic deposition in coronary arteries; these plaques are usually subocclusive and are typically stable. Clinical symptoms of stable angina occur when the oxygen demand of heart tissue cannot be adequately supplied by the coronary artery blood flow and is characterized by transient episodes of chest pain precipitated by exercise or increased activity; the pain is usually resolved with rest or nitroglycerin.

In unstable angina, the plaque is unstable causing platelet aggregation and activation of the coagulation systems, resulting in the formation of a platelet-rich thrombus (white thrombus). A thrombus rich in fibrin and erythrocytes (red thrombus) may evolve and extend up- or down-stream in the artery [15]. Extensive local thrombosis rich in platelets will result in episodic flow-limiting coronary stenosis and myocardial ischemia associated symptomatically with unstable angina or with necrosis that characterizes non-Q wave MI (see Fig. 3). If the plaque ruptures and thrombosis is extensive and rich in fibrin, the coronary artery may fully occlude and result in Q-wave MI (Fig. 3). If oxygen is not restored to the myocardium within 10 to 15 min, myocyte cell death and loss of membrane integrity occur. Infarct size and location are important in determining the clinical course and prognosis of acute MI.

3.3. MI interventions

Fig. 3 indicates that acute coronary syndrome patients can be grouped by their ECG findings. The ECG is important for guiding therapy because ST elevation patients generally have thrombus that is rich in fibrin [16]. For this reason, early fibrinolytic therapy using IV streptokinase or tissue plasminogen activator is efficacious in ST elevation patients. In contrast, the majority of patients with non-ST elevation MI (Fig. 3) have clots that are platelet rich [16]. Therefore therapies in non-ST patients are aimed at inhibiting platelet aggregation. A change in the conformation of the glycoprotein (GP) IIb/IIIa receptor is the final step in platelet aggregation and has been a primary therapeutic target of inhibition. POC testing for biochemical markers are particularly important for determining the care and improving the outcome of non-ST elevation patients.

The goal of therapeutic and mechanical interventions for acute coronary syndrome patients is to reduce morbidity and mortality by opening the occluded artery, thereby limiting infarct size and controlling complications. Because half of MI deaths occur within one hour of symptom onset, and the effectiveness of reperfusion strategies depends on early intervention, it is crucial to identify high-risk cardiac patients rapidly so that lifesaving treatment can be initiated. Typically, nitroglycerin is administered to relax smooth muscles leading to vasodilatation. Aspirin, considered to be the standard of care in all MI patients, inhibits platelet aggregation by irreversibly blocking the action of cyclo-oxygenase. Heparin acts at multiple sites in the coagulation cascade to inhibit clot formation.

Mechanical intervention usually consists of cardiac catheterization and percutaneous coronary intervention (PCI) or angioplasty. Along with PCI, placement of a stent(s) to sustain patency is also very common.

4. Biochemical markers for monitoring cardiac injury

4.1. General

4.1.1. The ideal cardiac marker

The characteristics of the ideal marker are summarized in Table 1. Unfortunately a single marker that satisfies all these characteristics does not exist so use of a profile of cardiac markers is necessary.

4.1.2. Quantitative vs. qualitative data

The assessment of myocardial necrosis and acute cardiac ischemia utilizes cut-points, which provide a context in which qualitative testing for cardiac markers is feasible. Quantitative assays may vary by 30-fold, so use of a qualitative assay with an appropriate cutoff may avoid discord between POC and the quantitative main lab assay. The feasibility of qualitative testing may be limited, however, because the positive cut-point for qualitative assays are prespecified and may vary several fold from corresponding quantitative methods. The prespecified cutoffs for qualitative assays do not allow adjustment should a lower or higher value be appropriate in the future. Importantly, when using
quantitative and qualitative assays, the cutoffs must be clinically evaluated to assure that there is no discord in results between the methods. There is overall general agreement that quantitative assays are preferred, particularly for monitoring the release (rise) and clearance (fall) of cardiac markers. Other applications such as risk stratification, reperfusion assessment and prognosis generally require continuous (numerical) data.

4.1.3. Serial sampling

Biochemical markers indicating myocardial necrosis require serial sampling to optimize diagnostic performance [17,18]. Markers of ischemia and necrosis require serial monitoring because their concentrations change rapidly in response to clinical events and they have a relatively short half-life in blood. It is critically important to place cardiac markers in the temporal context of clinical symptoms and signs. This is a substantial advantage for POC testing where availability of the biochemical marker is in the time frame when caregivers are focusing on the individual patient, particularly in the ED. Thus POC testing potentially saves physician’s time, allows high-risk patients to be treated more rapidly, and allows low-risk patients to be released in more timely fashion.

4.2. Markers of early myocardial ischemia

4.2.1. Background

The release of currently available myocardial markers into circulation is believed to require tissue necrosis, whereas the assessment of cardiac ischemia before or in the absence of cell death is always an important component of clinical decision-making. However, the diagnosis of cardiac ischemia is difficult and there is no set of reference criteria or guidelines for cardiac ischemia. Therefore, detection of cardiac ischemia depends on individual physician gestalt, which incorporates clinical assessment and imperfect tools including the ECG, echocardiography and imaging studies [19]. A sensitive biochemical marker of ischemia would be clinically useful particularly if available at POC.

4.2.2. Ischemia modified albumin (IMA)

A biochemical marker termed “ischemia modified albumin” (IMA) has been identified that evidently results from changes to the N-terminus of albumin caused by ischemia [20,21]. The scientific basis of this marker is that the low pH resulting from ischemia causes two biochemical events: a structural modification to the N-terminus of albumin, and release of copper from the protein ceruloplasmin, which binds with high affinity to the N-terminus of the intact albumin. A test for IMA monitors the binding between serum albumin and the transition metal cobalt [22]. This assay, termed the Albumin Cobalt Binding (ACB™) Test (Ischemia Technologies, Inc., Denver, CO) is based on observations that the binding of cobalt is reduced in serum from acute coronary syndrome patients [21]. Other information regarding IMA and myocardial ischemia is listed in Table 2.

4.3. Markers of myocardial infarction (MI)

4.3.1. Myoglobin

Table 2 shows characteristics of myoglobin, a heme protein that is located in the cytoplasm of both cardiac and skeletal muscle cells [23]. Myoglobin is the earliest appearing biochemical marker that is routinely available for assessment of the acute coronary syndrome patient. Meta-analysis has shown that the clinical sensitivity of myoglobin exceeds 90% with serial sampling at presentation and then 2 to 6 h later [24]. However, because myoglobin’s amino acid sequence is the same in both cardiac and skeletal muscle, clinical specificity is compromised due to increased values with skeletal muscle injury. Also, patients with renal insufficiency have elevated myoglobin levels due to decreased clearance. There are a number of studies that have examined the negative predictive value (NPV) of myoglobin, finding satisfactory high values in the range of 96% when chest pain onset is acute. Not all clinicians are convinced that myoglobin measurements contribute significantly to the assessment of acute coronary syndrome patients. However evidence from the CHECKMATE study clearly indicates that myoglobin significantly contributes to other markers for predicting outcome [25]. To optimize clinical utility, myoglobin results must be available within 30 min of the request. The major method used for myoglobin measurement is two-site immunoassay.

4.3.2. Creatine kinase-MB (CK-MB) isoenzyme

Cytoplasmic CK is dimeric, composed of M and/or B subunits that associate to form CK-MM, CK-MB, and
Table 2
Biochemical markers of the acute coronary syndromes.

<table>
<thead>
<tr>
<th>Biochemical Marker</th>
<th>Point of Care Test Available?</th>
<th>Molecular Weight, g/mole</th>
<th>Cardiac Specific?</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Duration of Elevation</th>
<th>Diagnostic Performance &amp; Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial Ischemia Markers</strong></td>
<td></td>
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</tr>
<tr>
<td>Ischemia Modified Albumin</td>
<td>Not at present</td>
<td>65,000</td>
<td>Unknown</td>
<td>Biochemical marker would be helpful because myocardial ischemia is difficult to diagnose</td>
<td>No ischemia “gold standard”, agreed reference clinical standard, or biochemical test for myocardial ischemia.</td>
<td>Preliminary data suggests 6–8 hours</td>
<td>Clinical trials pending</td>
</tr>
<tr>
<td>Glycogen</td>
<td>No</td>
<td>177,000</td>
<td>Yes</td>
<td>Biochemical marker would be helpful because myocardial ischemia is a difficult diagnosis</td>
<td>Inability to reproduce initial results.</td>
<td>8 hours</td>
<td>Initial data promising, but unable to be reproduced.</td>
</tr>
<tr>
<td>Phosphorylase-BB</td>
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<tr>
<td><strong>Myocardial Necrosis Markers</strong></td>
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<tr>
<td>Myoglobin</td>
<td>Yes</td>
<td>18,000</td>
<td>No</td>
<td>High Sensitivity and negative predictive value. Useful for early detection of MI and reperfusion.</td>
<td>Low specificity in presence of skeletal muscle injury and with renal insufficiency. Rapid clearance after necrosis.</td>
<td>12–24 hours</td>
<td>2–6 hours after presentation: Sensitivity: 90% (CI**: 88–93%) Speciﬁcity: 86% (CI: 85–87%) Negative Predictive Value: 96% Two serial values above 99th percentile of control reference population in the setting of ischemia is benchmark for myocardial necrosis.</td>
</tr>
<tr>
<td>CK-MB, mass assays</td>
<td>Yes</td>
<td>85,000</td>
<td>Yes</td>
<td>Ability to detect reinfarction. Large clinical experience. Previous “gold standard” for myocardial necrosis</td>
<td>Lowered speciﬁcity in skeletal muscle injury.</td>
<td>24–36 hour</td>
<td>Two serial values above 99th percentile of control reference population in the setting of ischemia is benchmark for myocardial necrosis.</td>
</tr>
<tr>
<td>Cardiac Troponin T</td>
<td>Yes</td>
<td>37,000</td>
<td>Yes</td>
<td>Tool for risk stratification. Detection of MI up to 2 weeks. High speciﬁcity for cardiac tissue</td>
<td>Not an early marker of myocardial necrosis. Limited ability to detect reinfarction.</td>
<td>10–14 days</td>
<td>A single value above 99th percentile of control reference population is surrogate of myocardial necrosis in setting of myocardial ischemia.</td>
</tr>
<tr>
<td>Cardiac Troponin I</td>
<td>Yes</td>
<td>23,500</td>
<td>Yes</td>
<td>Tool for risk stratification. Detection of MI up to 7 days. High speciﬁcity for cardiac tissue</td>
<td>Not an early marker of myocardial necrosis. No analytical reference standards. Limited ability to detect reinfarction</td>
<td>4–7 days</td>
<td>A single value above 99th percentile of control reference population is surrogate of myocardial necrosis in setting of myocardial ischemia.</td>
</tr>
</tbody>
</table>

* Time of first increase for the markers are 1–3 h for myoglobin, 4–6 h for CK-MB mass, 3–4 h for cardiac troponin T, and 4–6 h for cardiac troponin I.

** CI = 95% Confidence interval
CK-BB isoenzymes. In patients having significant myocardial disease, the CK-MB isoenzyme comprises approximately 20% of the total CK in this tissue. CK-MB is diagnostically sensitive for myocardial injury, however skeletal muscle has higher total CK activity per gram of tissue that may be comprised of up to 3% CK-MB. This potentiates compromised diagnostic specificity, particularly in patients with concomitant myocardial and skeletal muscle injury. To confer greater cardiac specificity to CK-MB measurements, a CK-MB “Index” is frequently calculated according to the following equation:

\[ \text{CK-MB Index} = 100\% \times \frac{\text{CK-MB}}{\text{CK-total}} \]

Some authors suggest that CK-MB Index values exceeding 2.5% are associated with a myocardial source of the MB isoenzyme; however, a recent review shows that the index is reportedly as low as 2% and as high as 5% depending on the variability of both the numerator and denominator [7]. CK-MB mass assays were considered the “gold standard” for MI diagnosis through the 1980s until about 1995, having a sensitivity of 96.8% (95% CI: 95–98%) and specificity of 89.6% (95% CI: 87–92%; [26]). Although CK-MB has lost its “gold standard” status to troponins (Table 2), CK-MB mass measurement is still a sound tool useful in the evaluation of MI [18].

The first rise in CK-MB after MI occurs 4 to 6 h after onset of symptoms, but serial sampling over a period of 8 to 12 h is required for high sensitivity [23]. Thus, despite excellent clinical performance, CK-MB is not an early marker and tissue specificity may be an issue.

POC methods utilize two-site immunoassays, or so-called “mass” assays. CK-MB mass assays are automated, highly sensitive (<1 μg/L) and specific, have a rapid turnaround time (as low as 7 min). Recently, the American Association for Clinical Chemistry (AACC) proposed a recombinant CK-MB material for standardization of CK-MB mass assays [27].

4.3.3 Proteins of the troponin complex

Fig. 4 shows that the troponin complex is located on the thin filament of striated muscle and consists of three sub-units: Troponin (Tn) T a binding protein which attaches the troponin complex to tropomyosin; TnI which modulates the interaction of actin and myosin by acting as an inhibitor of actomyosin ATPase activity; and TnC, the calcium-binding subunit of the troponin complex. Different isoforms encoded by separate genes are found in cardiac and skeletal muscle for both TnT and TnI muscle; the cardiac forms are designated cTnT and cTnI, respectively. Amino acid differences allow production of antibodies that specifically recognize the cardiac forms. Because TnC has an identical amino acid sequence in both skeletal and cardiac tissues, it has no potential as a cardiac specific marker.

As indicated in Table 2, cTnT and cTnI are markers of cardiac necrosis and require several hours to rise above detectable limits after onset of acute ischemia. For this reason troponin is not an early marker of necrosis and sampling for diagnostic use with high sensitivity and specificity requires specimen collection at patient presentation, at 6 to 9 h and at 12 to 24 h if clinical suspicion is high and earlier results were negative [18]. Table 2 indicates that elevation of both cTnI and cTnT after necrosis is prolonged; this allows their utilization for diagnosis many days after MI has occurred. The trade-off is that the precise pattern of troponin release is somewhat unpredictable, so cTnT and cTnI are less useful for assessing reocclusion or for infarct sizing (see later section).

Although the nature of the troponin complex released after myocardial injury is not fully characterized, several reports suggest that a large portion of TnI enters circulation as TnC-cTnT-cTnI and cTnI-TnC complexes, with only a small portion of TnI circulating in a free form [28]. Several studies have examined the degradation of the troponins both inside the cell and in circulation [29]. The released forms of cTnI and cTnT degradation in circulation after MI are incompletely understood at present, but may have clinical importance.

Standardization of cTnI assays is a substantial issue in laboratory medicine. This problem is complicated because there are both systematic (reference value) and assay specific (antibody) issues. Systematic issues take the form of value assignment for a reference material; assay specific differences result because different cTnI epitopes are targeted by the various assays. This situation is far less for cTnT because all of the commercial assays are produced by a single manufacturer. At present there can be a 30-fold difference between cTnI assays, which may be resolved, in part, by defining a reference material [30]. The AACC has formed a committee for addressing cTnI standardization [30].

5. Clinical applications of cardiac markers

Measurement of cardiac markers, alone and in combination, is a standard of care for the assessment, diagnosis,
monitoring, risk stratification, prognosis and guidance of therapy for patients within the continuum of acute coronary syndromes (Fig. 1). Presently available biochemical markers indicate necrosis, and cTnT and cTnI have become surrogates for cell death [18]. However markers of other processes fundamental to the acute coronary syndromes would also be useful. Plaque instability/disruption and platelet activation are common physiologic features in the acute coronary syndromes; therefore markers reflecting plaque stability and activated or “angry” platelets would have great clinical potential. Inflammation markers such as C-reactive protein have demonstrated use for assessment of long-term risk, but also may have a role in assessing the suspected acute coronary syndrome patient [31]. Ischemia is the central physiologic process; initial data on IMA are promising, and testing for acute cardiac ischemia would also have an important role in clinical settings. The evidence for markers of cardiac necrosis including myoglobin, CK-MB and troponin is well established [17,18,23], and utilization of these markers will be the focus of this section.

5.1. Assessment of cardiac ischemia

Clinical assessment of (possible) cardiac ischemia and unstable angina is extremely difficult because patients may present with a variety of nonspecific signs and symptoms including shortness of breath, jaw pain, indigestion, nausea, vomiting, and so on. Further, there is no “gold standard” marker for cardiac ischemia; the ischemia diagnosis is based on clinical judgement. Yet the assessment of cardiac ischemia is critically important because these patients are at substantial risk for subsequent cardiac events.

A simple, rapid diagnostic test for cardiac ischemia would be very valuable for the work-up of patients suspected of having cardiac ischemia. The IMA test is promising for this use [22]; conditions for altering albumin’s amino terminus region, and therefore albumin cobalt binding, can occur within minutes of an ischemic event due to induced endothelial and extracellular hypoxia, acidosis, free radical injury, and sodium and calcium pump disruptions [32,33]. Although there is no agreed-upon reference for assessing myocardial ischemia, IMA testing may add useful information into clinical acumen, imaging techniques, and stress testing.

5.2. Diagnosis of myocardial infarction

For over 20 yr, the definition and diagnosis of MI were based on modified World Health Organization (WHO) criteria in which patients must meet at least two of three conditions [34]: typical symptoms of ischemia, a diagnostic ECG, and a characteristic rise and fall in “enzymes”, later interpreted as CK-MB. There are a number of issues with these MI criteria. Although symptoms are generally what cause individuals to seek care, typical clinical signs of ischemia are absent in 1/3 of MI patients [35]. The ECG, as indicated in Fig. 3, is essential for assessment and classification of acute coronary syndrome patients. However only 45% of patients who are eventually diagnosed as having MI present with a diagnostic ECG [36,37]. Insensitivity of the ECG for predicting outcome was underscored in a recent analysis of 350,000 MI patients [38]. This study demonstrated that 19.2% of MI patients having a normal initial ECG, and 27.5% showing nonspecific ECG changes either died or experienced a potentially life threatening adverse event [38]. Clearly the best approach would be to integrate ECG characteristics with biochemical cardiac markers [39]; however the best strategy for combining the ECG and cardiac markers for immediate risk stratification remains to be further elucidated [39]. When the WHO criteria were defined in 1979, “enzyme” biochemical markers included total CK activity, lactate dehydrogenase (LDH), and aspartate transaminase (AST). In the years after the definition of WHO criteria, a typical rise and fall in CK-MB became the accepted benchmark from the 1980s to ~1995 [23]. An update in MI definition was needed since entering “the era of troponin”.

Development of sensitive and specific assays for CK-MB mass, cTnT, cTnI and myoglobin, as well as evolution of precise imaging technologies have prompted reevaluation of the established MI definition by a joint committee of the European Society of Cardiology (ESC) and the American College of Cardiology (ACC; 18). The resulting ESC/ACC consensus presented a new definition of myocardial necrosis in which elevations of the troponins or CK-MB are the cornerstone. Biochemical criteria for detecting myocardial necrosis in the setting of myocardial ischemia are: (a) maximal concentration of cTnT or cTnI exceeding the 99th percentile of a reference control group on at least one occasion during the first 24 h, (b) “maximal” value of CK-MB exceeding the 99th percentile of the values for a reference control group on two successive samples, or maximal value exceeding twice the upper limit of normal for the specific institution on one occasion during the first 24 h [18]. Further the ESC/ACC committee recommended that imprecision (CV) at the marker cutpoint should be ≤ 10%.

The following criteria satisfies the diagnosis of acute, evolving, or recent MI [18]: (a) typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following: (i) ischemic symptoms; (ii) development of pathologic Q waves on the ECG; (iii) ECG changes indicative of ischemia (ST segment elevation or depression); or (iv) coronary artery intervention (e.g., coronary angioplasty); (b) pathologic finding of an acute MI.

These new criteria are particularly significant because many patients who were previously diagnosed as having severe stable or unstable angina will now have the diagnosis of MI, with all the associated sociological, epidemiologic, financial, and other implications. The incidence of MI will increase by an estimated 15 to 30%, which translates into about 100,000 to 200,000 more MI cases each year.
Importantly, the ESC/ACC report stressed serial sampling for cardiac markers, recommending sampling upon presentation, at 6 to 9 h, and again at 12 to 24 h if the earlier samples were negative and the clinical index of suspicion is high [18].

5.3. Reocclusion (reinfarction)

Reocclusion occurs in up to 10 to 20% of MI patients and is an important determinant of early and late morbidity and mortality [40]. Criteria commonly used to identify recurrent MI include prolonged chest pain, new ECG changes, and biochemical markers. Because cTnT and cTnI have an extended half-life in blood and their time course is somewhat unpredictable, markers with a shorter more predictable time-course such as CK-MB are utilized to more clearly indicate timing of the clinical event.

The rapid turnaround offered by POC measurements could develop into an important component of care for these patients because benefits from the interventional options for reocclusion are time-sensitive.

5.4. Noninvasive assessment of reperfusion

As discussed earlier, ST elevation or new left bundle branch block on the ECG guides the use of reperfusion therapy that includes “clot busting” thrombolytic drugs such as streptokinase and tissue plasminogen activator. The historical “gold standard” for assessing the success of reperfusion therapy is epicardial perfusion as assessed by angiography using the following grading scale [41]: TIMI 0: no anterograde flow beyond the point of occlusion, TIMI 1: penetration without perfusion, TIMI 2: partial perfusion, and TIMI 3: complete perfusion. Successful reperfusion is defined as TIMI 2 or 3 flow in some studies, but success for optimizing outcome is usually considered establishment of TIMI 3 flow in the infarcted artery [42].

It is noteworthy that while establishing epicardial patency has been the goal historically, there has been an important shift “downstream” to redefine the open-artery hypothesis to include not only rapid epicardial patency, but also restored microvascular flow and tissue perfusion [43]. Biochemical cardiac markers and other noninvasive biomarkers may prove to be useful complements to angiography for evaluating successful (re)perfusion and for evaluation of new reperfusion regimens [43].

Thrombolytic therapy is successful in achieving TIMI 3 patency in only 60 to 70% of patients [42]. Because cardiac catheterization is invasive, expensive and is not available at every medical center, strategies that include biochemical markers have been developed to assist clinicians in determining patients whose infarct-related artery patency remains suboptimal, and who may benefit from immediate, alternative interventions. A model that combines clinical variables with myoglobin and CK-MB data in samples collected just before thrombolytic therapy, and then at 60 and 90 min later shows substantial promise [43,44]. Myoglobin due to its rapid “washout” after thrombolysis, CK-MB and perhaps also troponin may be important for reperfusion monitoring [44]. POC testing will be critical for monitoring the success of reperfusion because a rapid turnaround time is needed to determine if thrombolysis has not been successful, so that alternate care strategies such as PCI or coronary artery bypass surgery must be considered to fully “open the artery” and improve outcome.

5.5. Risk stratification

The discovery that cTnT and cTnI predict increased risk for an adverse outcome in acute coronary syndrome patients must be considered one of the most important findings in laboratory medicine over the past decade. A number of outcome studies demonstrating this increased risk have been analyzed in systematic reviews. One meta-analysis that included results of 2,847 unstable angina patients, with a median follow-up duration of 30 days, showed that a positive cTnT was associated with cumulative odds ratio (OR) of 2.7 (95% CI: 2.1–3.4, $\chi^2 = 66$) for the risk of AMI and cardiac death [45]. For positive cTnI results, this meta-analysis [45] yielded a cumulative OR of 4.2 (95% CI: 2.7–6.4, $\chi^2 = 42$) for the risk of AMI and cardiac death in 1,901 unstable angina patients with a median follow-up duration of 42 days. A separate systematic review examined the association of positive cTnT or cTnI with 30-day death or MI in a total of 18,982 acute coronary syndrome patients from 21 studies [46]. This larger analysis showed that positive troponin indicated greater risk with a cumulative OR of 3.44 (95% CI: 2.94–4.03). This study [46] also examined troponin results based on ECG findings; a positive troponin in ST elevation patients was associated with a 2.86-fold (95% CI: 2.35–3.47) increased risk of 30-day death or MI; in the non-ST elevation group, risk of death or MI was increased 4.93-fold (95% CI: 3.77–6.45). Further, a study that examined troponin and mortality or MI at 1-yr also found a higher risk profile for troponin-positive patients at this endpoint [47]. The consistent message is that troponin positive acute coronary syndrome patients are at 3- to five-fold increased risk of death or MI in the 30 to 40 days following the index event, and that this higher risk extends to longer times.

Timing of sampling for troponin measurement is important for risk stratification. Although troponin measurement at presentation provides the most information, measurements must be performed in samples collected at 8 to 16 h because this strategy show a significant improvement in the ability to risk stratify [47]. Also, sampling at presentation and then 8 to 16 h later helps to avoid possible methodological differences among troponin assays.

This strong evidence for risk stratification indicates that testing for cTnT and cTnI are appropriate for triage and decisions relating to hospital admission and perhaps interventions, such as PCI [48,49]. Development of POC assays
for cTnT and cTnI have facilitated delivery of this important information, so that caregivers can appropriately stratify high-risk patients. Most importantly, clinical management and guidance of therapy for acute coronary syndrome patients based on risk stratification by biochemical markers “is an idea whose time has come” [50].

5.6. Guidance of therapy and intervention

The strong evidence that positive troponin measurements translate to increased risk in acute coronary syndrome patients has led to guidelines for unstable angina and non-ST elevation that designate troponin positive patients as high risk. These patients should receive medications such as aspirin, beta blockers, nitrates, and either an early invasive or early conservative strategy of care [48,49]. This translation of troponin positivity to increased risk has been confluent with development of novel therapeutics targeting platelet physiology. These therapeutics are aimed at improving the outcome of acute coronary syndrome patients, particularly those having unstable angina and non-ST elevation MI. Because these drugs are frequently expensive and definitive benefit is difficult to demonstrate, directing therapy at high-risk patients identified with biochemical markers is a logical extension of knowledge.

Identifying patients with unstable coronary artery disease who may benefit from LMW heparin by use of cTnT measurements was examined in the FRISC study [51]. In patient groups having positive cTnT (≥ 0.1 ng/mL), those receiving placebo had a significantly higher incidence of death and/or MI compared to patients randomized to LMW heparin. On the other hand, patient groups that were cTnT negative (<0.1 ng/mL) were both at low risk, and showed no difference in outcome whether they received placebo or LMW heparin [51].

An important focus in cardiovascular therapeutics has been development of agents that are antagonists for the platelet GP IIb/IIIa receptor. These agents inhibit conformational changes in the GP IIb/IIIa receptor, which is the final step in platelet activation. Blockage of this receptor prevents platelets from becoming activated (angry) and potentiating thrombosis. The CAPTURE study included unstable angina patients who were randomized to receive either the GP IIb/IIIa receptor inhibitor abciximab or placebo [52]. Patients for whom cTnT was negative (<0.1 ng/mL) showed a similar low incidence of death or MI for both in-hospital and on 6-month follow-up whether they received placebo or the GP IIb/IIIa inhibitor. Consistent with risk stratification data, patients receiving placebo who were cTnT positive had a significantly higher rate of adverse events. Importantly, however, the cTnT positive patients who received the GP IIb/IIIa inhibitor had a significantly lower rate of death or MI, which was similar to both cTnT negative groups. Thus, cTnT measurements identified a high-risk group that benefited from administration of the drug. Another GP IIb/IIIa inhibitor, tirofiban, was examined in the PRISM study [53]. This randomized trial also showed that cTnI and cTnT reliably identified high-risk patients who benefited from treatment. Further, it is of note that the only patients who benefited from tirofiban were troponin positive, strongly suggesting a central role in guidance of this therapy [53]. Finally, the PARAGON B study examined the association between troponin concentrations and a third GP IIb/IIIa inhibitor, lamifiban [54]. PARAGON B also found that cTnT identified patients who had better outcomes with drug treatment. Together these GP IIb/IIIa inhibitor studies included over 4500 patients and serve to validate utilization of troponin for identification of high-risk individuals and guidance of GP IIb/IIIa inhibitor therapy. These compelling data have prompted inclusion of troponin positivity into guidelines for identification and clinical management of high-risk unstable angina patients [48] and also for guiding management of patients with acute MI [49].

Unexpectedly, a trial of 7800 patients did not show benefit of a GP IIb/IIIa inhibitor in patients who were troponin positive or had ST depression. The GUSTO IV study included patients with a relatively low-risk profile that included only 5 min of chest pain and either ST changes or positive troponin [55]. However the substantial amount of data supporting the use of GP IIb/IIIa inhibitors in the medical management of high-risk acute coronary syndrome patients who are troponin positive remains valid [55].

POC testing for biochemical markers will be driven by the improvement in outcome attributed to earlier intervention with cardiac catheterization and PCI or GP IIb/IIIa inhibitors. For this reason the NHAAP has designated that time from order to availability (turnaround time) for cardiac markers should be 30 min, and that this time should become a quality indicator. POC testing for cardiac markers will become essential at most institutions to meet this criterion.

5.7. Prognosis (infarct sizing)

Assessing myocardial cell death after prolonged ischemia is a semiquantitative system according to the following four pathologic classifications: 1. microscopic, with focal necrosis; 2. small, <10% of the left ventricle; 3. medium, 10 to 30% of the left ventricle; and 4. large, >30% of the left ventricle [18]. However, clinically and for use in research trials where short-term mortality is relatively low, the use of biochemical marker curves for prognostication and predicting outcomes is most appropriate [18]. Since the 1970s, investigators have studied the use of markers for quantifying (sizing) myocardial necrosis [56,57], reasoning that the greater the extent of cardiac cell death, the worse the prognosis. The general approach has involved serially measuring markers in blood over hours and days after the clinical event and then mathematically modeling the release profile. By integrating the area of the time-release curve, calculating peak concentration, etc. it was possible to determine the quantity of myocardial tissue that was injured irreversibly.
Biochemical markers including α-hydroxybutyrate dehydrogenase, myoglobin, total CK and CK-MB have been used for infarct sizing and prognosis. Tradeoffs in sample timing and predictability of release has favored use of total CK and CK-MB, and their association and outcomes are well established [58,59]. Infarct size correlates closely with mortality as well as other prognostic indexes such as cardiac failure, ventricular function and arrhythmia [60]. Peak CK-MB value has been found to significantly correlate with 5-yr outcome [61]. In the setting of PCI, measured elevations in CK-MB have also demonstrated a strong association with outcomes [62].

POC testing of cardiac markers, mainly CK-MB, for use in prognosis may be performed in the intensive care unit, or in the post intervention areas.

6. Point of care testing

The main objective of POC testing, also known as near patient or bedside testing, is to provide quick accurate and precise results so that appropriate decisions and treatment strategies can be applied, leading to improved clinical outcome. POC testing achieves STAT by reducing the time spent in test ordering, specimen transport to a remote laboratory, and data reporting. Another “psychologic” advantage of POC testing is the fact that data are owned by the clinical team and there is no interruption of the clinical decision-making process while awaiting results from the central laboratory. POC tests are usually performed in the ED, chest pain evaluation centers (CPECs), and intensive care units. Although POC testing is more expensive than laboratory testing, it is believed to reduce hospital stay, improve adherence to treatment and reduce complications.

The development of sophisticated analytical systems over the past decade enabled a wide range of tests to be performed quickly and simply without the need for relatively complicated laboratory equipment.

Two broad types of technology support POC testing: small top analyzers, and hand held devices. Although the assay principles of both POC devices and laboratory equipment are essentially similar, POC devices have been modified to reduce operator errors and provide faster results. Bench top analyzers are smaller versions of laboratory equipment in which operator-dependent steps have been automated; however hand held devices have been developed using microfabrication techniques. Although, hand held devices appear simple, they are equipped with complex systems that perform several tasks, e.g., separating cells from plasma, reading analyte signal, data processing, and system interface. The characteristics of the currently available POC technologies for measurement of cardiac markers are displayed in Table 3. Different strategies of cardiac POC tests are summarized below.

6.1. POC assays for cardiac markers

Immunooassay is the common analytic strategy for POC measurement of cardiac markers. Although the assay principles are similar to central laboratory testing, POC devices and technologies have been modified to simplify performance, reduce the chance of operator error, use a whole blood sample matrix, provide faster results, and streamline quality control/assurance processes. POC devices are designed for simple operation, but are actually complex systems that perform several tasks, such as separating cells from plasma, apportioning the proper amount of sample, reading analyte signal, data processing, and system interface. The characteristics of the currently available POC technologies for measurement of cardiac markers are displayed in Table 3. Different strategies of cardiac POC tests are summarized below.

6.1.1. Dry chemistry tests
6.1.1.1. Roche Cardiac ™ and Roche Cardiac™ M tests (Roche Diagnostics Corp., Indianapolis, IN)

Both assays employ immunochromatography with two antibodies that bind to different molecular epitopes in a single-step “sandwich” immunooassay format. One of the antibodies is biotinylated, to facilitate capture with polystreptavidin, and the second is conjugated to gold particles for detection. The cTnT assay uses the same pair of anti-cTnT antibodies that are used for measurement with Roche’s Elecsys® laboratory system. This facilitates harmonization between POC and central lab results.

Measurements are initiated by addition of heparinized whole blood to the sample well. Capillary action combined
Table 3
Characteristics of Currently Available Cardiac POC Assays

<table>
<thead>
<tr>
<th>Device</th>
<th>Cardiac Marker</th>
<th>Suggested Cut-off</th>
<th>Manufacturer’s Claim</th>
<th>Time (min)</th>
<th>Specimen (Type &amp; volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Cardiac T® Rapid Assay</td>
<td>cTnT</td>
<td>0.1 ng/mL*</td>
<td>Myocardial damage is detected</td>
<td>12</td>
<td>150 µL heparin whole blood</td>
</tr>
<tr>
<td>Roche Cardiac™ M Rapid Assay</td>
<td>Myoglobin</td>
<td>Male: 76 ng/mL Female: 64 ng/mL</td>
<td>Above normal range</td>
<td>8</td>
<td>150 µL heparin whole blood</td>
</tr>
<tr>
<td>¶Cardiac STATus™ device (Spectral Diagnostics)</td>
<td>CK-MB</td>
<td>5 ng/mL (Abbott method)</td>
<td>Aid in the diagnosis of cardiac ischemia</td>
<td>15</td>
<td>200 µL serum or heparinized whole blood or plasma</td>
</tr>
<tr>
<td>¶Cardiac STATus™ device (Spectral Diagnostics)</td>
<td>Myoglobin</td>
<td>50 ng/mL (Behring Diagnostics method)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¶Cardiac STATus™ device (Spectral Diagnostics)</td>
<td>cTnI</td>
<td>1.5 ng/mL (Dade Stratus Method)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha Dx™ POINT-OF-NEED System (First Medical)</td>
<td>Total CK mass</td>
<td>190 ng/mL</td>
<td>Aid in the diagnosis of AMI</td>
<td>18</td>
<td>1.5 mL EDTA whole blood or serum</td>
</tr>
<tr>
<td>Alpha Dx™ POINT-OF-NEED System (First Medical)</td>
<td>CK-MB</td>
<td>7.0 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha Dx™ POINT-OF-NEED System (First Medical)</td>
<td>RI (%)</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha Dx™ POINT-OF-NEED System (First Medical)</td>
<td>Myoglobin</td>
<td>100 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha Dx™ POINT-OF-NEED System (First Medical)</td>
<td>cTnI</td>
<td>0.40 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratus® CS STAT Fluorometric Analyzer (Dade Behring, Inc.)</td>
<td>CK-MB</td>
<td>3.5 ng/mL</td>
<td>All markers: Aid in the diagnosis of AMI</td>
<td>14 min to first result, 4 min for each additional result</td>
<td>Whole blood (lithium or sodium heparin): 3 mL</td>
</tr>
<tr>
<td>Stratus® CS STAT Fluorometric Analyzer (Dade Behring, Inc.)</td>
<td>Myoglobin</td>
<td>Male: 98 ng/mL; Female: 56 ng/mL</td>
<td></td>
<td></td>
<td>Plasma (lithium or sodium heparin): 200 µL for first test, 100 µL for each additional test on the same run</td>
</tr>
<tr>
<td>Stratus® CS STAT Fluorometric Analyzer (Dade Behring, Inc.)</td>
<td>cTnI</td>
<td>0.06 gn/mL</td>
<td>cTnI: Risk Stratification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triage® Cardiac Panel (Biosite Diagnostics)</td>
<td>CK-MB</td>
<td>10.0 ng/mL</td>
<td>Aid in the diagnosis of MI</td>
<td>~15</td>
<td>250 µL heparinized whole blood or plasma</td>
</tr>
<tr>
<td>Triage® Cardiac Panel (Biosite Diagnostics)</td>
<td>Myoglobin</td>
<td>170.0 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triage® Cardiac Panel (Biosite Diagnostics)</td>
<td>cTnI</td>
<td>1.0 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¶Myoglobin (Response Biomedical)</td>
<td>Myoglobin</td>
<td>–</td>
<td></td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* Values <0.05 ng/mL indicate negative TnT; values between 0.05 to <0.1 ng/mL indicate low TnT and test should be repeated within 1 hr with a fresh blood sample; values between 0.1–2.0 ng/mL indicate myocardial damage; values >2.0 ng/mL indicate massive myocardial damage.

¶ This assay yields qualitative results.

† This assay is not commercially available at press time.
with filtration separates cellular constituents from the plasma, which solubilizes the antibodies and other reagents. The two antibodies form sandwich complex with any cTnT or myoglobin present. The sandwich complex migrates with plasma along the test strip by capillary action until it reaches polystreptavidin, which is immobilized in a thin line across the read window of the test strips. At this point the polystreptavidin captures the biotinylated end of any immune sandwiches, serving to concentrate the complexes. Further flow of plasma through the read window carries unreacted anti-cTnT or anti-myoglobin antibodies conjugated with gold particles past the polystreptavidin read window, where they combine with a fragment of cTnT or myoglobin bound to an anchor protein immobilized in the QC test line. The concentrated gold particles produce a reddish purple line(s) that is interpreted visually for qualitative tests (CARDIAC T only), or quantified by the Roche CARDIAC™ Reader. The on-board positive QC test line is integral to each rapid assay strip and must be formed for the test result to be valid. The intensity and rate at which the colored line develops is directly proportional to the concentration of cTnT or myoglobin in the blood.

6.1.1.2. Cardiac STATus™ device (Spectral Diagnostics Inc., Toronto, Canada)

The STATus™ assay system uses chromatographic solid-phase immunoassay technology to provide qualitative results for a testing panel that includes myoglobin, CK-MB mass, and cTnI. A second device for qualitative measurement of cTnI is also available.

Heparinized whole blood, heparinized plasma or serum specimens may be used. After initial separation of the plasma fraction from the cellular blood components by a glass fiber fleece, the migrating plasma dissolves a buffer and solubilizes the reagent antibodies. The myoglobin, CK-MB or cTnI analyte in the patient sample reacts with dye-labeled antibodies that are analyte-specific. The [dye-labeled antibody/analyte] complexes migrate by immunochromatography to the detection zone, where capture antibodies directed at different epitopes on the myoglobin, CK-MB or cTnI molecules immobilize the complexes at different positions, each in line formation. Unbound labeled antibodies migrate out of the test area and are later captured by solid-phase anti-IgG antibodies in the control “CON” area, forming a fourth QC line. The appearance of control zones proper performance of the device including unimpeded plasma flow. If a band is present only in the “CON” area, the test result is read as negative. If no band is present in the “CON” area, the test should be considered invalid and another device must be run, regardless of the presence or absence of a band in the test area.

6.1.1.3. Triage® system for cardiac marker measurement (Biosite Diagnostics Inc., San Diego, CA)

The Triage® Cardiac system consists of two basic components, a wafer-shaped test cartridge that contains all necessary analytic reagents and the Triage® Meter. The system provides for quantitative measurement of a cardiac marker panel including myoglobin, CK-MB, and cTnI. Analytes are quantified simultaneously in respective one-step “sandwich” immunoassays using immunochromatography with fluorescence detection.

After dosing the cartridge with heparinized whole blood or plasma, the plasma and cellular constituents are separated by filtration. The plasma phase is delivered to areas on the cartridge containing dried immunoassay reagents specific for myoglobin, cTnI, or CK-MB measurement. During incubation the reaction mixture flows down a micro-capillary and comes in contact with immobilized antibodies, directed at different myoglobin, CK-MB or cTnI epitopes, that are oriented as different lines for each analyte in the detection zone. The binding of analyte labeled with fluorescent reagents in these zones is measured by a fluorometer in the Triage® Meter and is directly proportional to the concentration of the analytes being tested. The signal for each cardiac marker is compared to its specific analytical curve allowing quantitative measurement. Each of the myoglobin, cTnI, and CK-MB immunoassays has internal quality control zones to ensure their validity and accuracy.

6.1.2. The Alpha Dx™ POINT-OF-NEED™ System™ (First Medical, Mountain View, CA)

This innovative system integrates automated solid-phase sandwich immunoassay technology with fluorescence detection. In comparison with a typical sandwich fluorescent immunoassay, the Alpha Dx system utilizes three antibodies: a fluorescein-labeled antibody, a solid phase capture antibody directed at a different epitope, and a fluorescent-labeled (Cy5) antifluorescein monoclonal antibody that is conjugated to dextran. The three-antibody system increases signal amplification and enhances assay sensitivity.

The system uses an EDTA whole blood sample to quantitatively measure a profile of cardiac markers that includes myoglobin, total CK mass, CK-MB mass, and cTnI. The test disc consists of four fluidically connected chambers: one for sample (or control) introduction, containing the fluorescein-labeled antibodies to the analytes; a second chamber for reaction, containing analyte-specific capture antibodies; a third for the fluorescently labeled antifluorescein dextran conjugate for detection, and a fourth wash chamber. Small magnetically activated stainless steel balls in the sample and label chambers facilitate reagent rehydration and mixing; the disc is spun to move fluids through the chambers. Each disc has three sets, or “zones” of fluidic chambers. One zone is used for the test sample and the others are for two levels of on-board quality control. Each disc also contains a waste ring and a hematocrit chamber where packed cell volume is measured and used to convert the measured whole blood sample data to matched serum results. In all assays, fluorescence intensity is proportional to the amount of bound label and consequently to analyte.
6.1.3. Compact desktop analyzer

The Stratus® CS STAT Fluorometric system (Dade Behring Inc., Glasgow, DE) uses two-site sandwich immunoassay technology and fluorometric detection in a solid-phase radial partition chromatography format for random-access quantitative measurement of CK-MB mass, myoglobin, and cTnI in lithium and sodium heparin whole blood or plasma samples. The test system employs monoclonal capture antibodies conjugated to dendrimers, which are polymers composed of polyamine groups characterized by uniformity in size and shape. Compared to earlier technology, dendrimers enhance presentation and functionality of the immobilized antibody on the system’s solid-phase, resulting in more efficient antigen capture. The cTnI-specific antibodies are identical to those used on the Dimension® system so there is analytical harmony between the POC and central lab systems. Also, the Stratus CS and Dimension antibodies bind both free and complexed cTnI.

The Stratus® CS analyzer uses on-board centrifugation to separate plasma from whole blood before the analytic phase; alternatively, heparinized plasma can be analyzed. During measurement, plasma is automatically pipetted onto the solid-phase where it reacts with an immobilized monoclonal antibody, linked to dendrimer, which recognizes a distinct epitope on the myoglobin, CK-MB, or cTnI analyte. Following incubation, a second monoclonal antibody, conjugated to alkaline phosphatase and recognizes a different antigenic site on the analyte, is pipetted onto the reaction zone. Detection and removal of unbound conjugated antibody are accomplished simultaneously by addition of the enzyme substrate and wash solution. Front surface fluorometry of the 4-methylumbelliferone enzyme product is used for quantitation.

6.2. Cardiac markers: why POC testing?

Addressing clinically focused questions is essential for achieving clinically relevant answers. The following are clinically based questions that focus on issues relevant to POC testing for cardiac markers.

Question 1. Is troponin important for identification of patients at increased risk of adverse outcomes?

Troponin results are definitely needed for appropriate risk stratification of patients suspected of acute coronary syndromes. Meta-analyses [45,46] provide strong and compelling evidence and the American College of Cardiology (ACC)/American Heart Association (AHA) Task Force issued practice guidelines for the management of patients with unstable angina and non-ST segment elevation MI [48]. For early risk stratification, these ACC/AHA recommendations state that “biomarkers of cardiac injury should be measured in all patients who present with chest discomfort consistent with acute coronary syndromes. A cardiac-specific troponin is the preferred marker... it should be measured in all patients. In patients with negative cardiac markers within 6 h of the onset of pain, another sample should be drawn in the 6- to 12-h time frame (e.g., at 9 h) after the onset of symptoms”.

Question 2. Is turnaround time (“vein-to-brain” or order to availability time) of biochemical markers (troponin) related to outcome for high-risk patients?

Studies have demonstrated that earlier treatment of high-risk acute coronary syndrome patients with GP IIb/IIIa inhibitors improves clinically important outcomes [55]. Also, early intervention with PCI improves outcomes [62]. Practice guidelines list positive biomarker (troponin) results as important, evidence-based determinants of high-risk [48, 49]. For these reasons the NHAAP has listed a “vein-to-brain” time of 30 min as a goal, and that order-to-availability time for biochemical markers should be quality indicator for clinical care.

Question 3. Does POC testing of biochemical markers improve availability (“vein-to-brain”) time?

Two studies comparing central lab vs. POC testing have shown that near patient assays markedly improved availability of troponin results by eightfold (median of 128 min vs. 15 min) [63] and 3.5-fold (72 min vs. 20 min) [64]. Transport time and need for centrifugation and processing are key components that delay availability of data with centralized laboratory testing. If the laboratory component exceeds 25% of the decision time, then POC testing should be implemented [64]. According to the NHAAP goal of 30 min, this translates to a total in-lab time of 7.5 min.

Question 4. Which cardiac markers should be included in POC testing? Does the availability of POC testing for cardiac markers affect the outcome and management of patients with suspected acute coronary ischemia?

Evidence answering this critical question was presented in “The Chest Pain Evaluation by Creatine Kinase-MB, Myoglobin, and troponin I” (CHECKMATE) study [25]. This study of 1005 chest pain patients without ST-segment elevation was designed to prospectively evaluate the ability of quantitative bedside measurement of: 1. a three-member panel including myoglobin, CK-MB and cTnI (My-MB-cTnI); 2. a two-member panel (MB-cTnI) and the local lab strategy for risk stratification of outcomes 30-day death and MI. With sampling at 0, 3, 6, 9 to 12 and 24 h, the My-MB-cTnI and MB-cTnI strategies identified positive patients earlier than the local lab strategy. Thus use of a multi-marker panel enables earlier treatment or intervention compared to the local lab strategy. Importantly, the My-MB-cTnI panel discriminated 30-day death better than either the MB-cTnI, or local lab strategies [25]. The My-MB-cTnI panel also performed better than a My-cTnI profile (personal communication: L. Kristin Newby, M.D., Duke University, Durham, NC). The My-MB-cTnI panel identified pos-
itive patients earlier, provided better risk stratification for mortality, and is therefore the panel of choice for assessing chest pain patients arriving in the ED with suspected non-ST elevation acute coronary syndromes.

7. Conclusions

POC testing based on important clinical outcomes is justified. Qualitative and quantitative POC testing devices are now available for myoglobin, CK-MB, cTnI, and cTnT. These assays use anticoagulated whole blood and have total analysis times <20 min. Eliminating transport of samples to the central laboratory, centrifugation and processing enables an order-to-availability time of 30 min. POC tests provide diagnostic performance that is similar to that of the central laboratory cardiac assays. Currently, most of POC tests measures a panel of cardiac markers with complementary diagnostic information. Harmony between values for the central laboratory and POC testing site remains an important issue.

As with any POC testing program, success of implementing POC cardiac markers depends on cooperation between the clinical staff, i.e., physicians and nurses, hospital administrators, laboratorians, and manufacturers. It seems clear that responsibility for POC testing must reside with the laboratory where involvement must include the selection of POC devices, education, training, maintenance and implementation of a quality assurance program with appropriate monitors. Proper implementation of POC testing provides the laboratorian with an excellent opportunity to work with clinicians and improve the outcomes of patients with the acute coronary syndromes—the world’s most lethal killer.

References


