Biochemical markers of the acute coronary syndromes

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The acute coronary syndromes represent a continuum of myocardial ischemia ranging from angina, reversible tissue injury \(\rightarrow\) unstable angina, frequently associated with minor myocardial damage \(\rightarrow\) myocardial infarction and extensive tissue necrosis. Historically, coronary artery disease assessment has been mainly binary, using WHO criteria of symptoms, electrocardiography, and biochemical markers. The creatine kinase-MB isoenzyme (CK-MB) has been a benchmark for markers, but it is not specific for myocardium. Cardiac-specific isoforms of troponin T and I have emerged as sensitive myocardial infarction (MI) indicators and, importantly, for risk stratification of acute coronary syndrome patients. In addition to markers of myocardial cell necrosis, markers of plaque disruption (C-reactive protein and serum amyloid A), “angry” platelets (P-selectin), ischemia (glycogen phosphorylase-BB isoenzyme), and the procoagulant state and thrombosis (soluble fibrin) have potential use. Also, CK-MB and myoglobin have been combined with clinical indicators for monitoring reperfusion after thrombolytic therapy. Biochemical markers will continue to be an important clinical adjunct for MI diagnosis, risk assessment, and reperfusion monitoring in the future.

Biochemical markers of myocardial injury will continue to play an essential role in global assessment and treatment of patients presenting within the spectrum of acute coronary syndromes, a term encompassing the continuum of acute myocardial ischemia ranging from angina through Q-wave myocardial infarction (MI).5 As indicated in Fig. 1, the continuum of acute coronary syndromes reflects the physiological process of acute myocardial ischemia and, perhaps most importantly from a clinical standpoint, a continuum of risk. For much of the past three decades, myocardial ischemia has been regarded as a binary phenomenon, i.e., MI or non-MI, using WHO recommendations that include fulfillment of at least two of the following three criteria: clinical symptoms suggestive of myocardial ischemia, electrocardiographic (ECG) changes, and an increase of serum biochemical markers. For the first criterion, careful assessment of clinical symptoms is obviously of paramount importance; however, symptoms can be nonspecific in up to one-third of patients, particularly in diabetics and the elderly, who most frequently present with atypical symptoms of ischemia (1). The second criterion, changes on the ECG, is a most important tool that should be performed quickly after presentation of the suspected MI patient because those having either ST-segment elevation \(\geq 1 \text{ mV in contiguous leads or symptoms or new left bundle branch block are candidates for immediate reperfusion therapy (2). However, the ECG is not a perfect instrument because its diagnostic sensitivity may be as low as 50% (3–6). The final WHO criterion involves monitoring the temporal change in biochemical markers of myocardial necrosis. In the past, enzyme activity was used as the marker; however in the future, measurement of proteins, some of which are enzymes, will become the standard. Observing the rise and fall of the biochemical marker creatine kinase-MB isoenzyme (CK-MB) has been termed the “gold standard” for the diagnosis of MI (5).

In symptomatic patients presenting with diagnostic ECG changes, biochemical markers have a limited role in the context of acute MI diagnosis, except for confirmation. On the other hand, biochemical markers are essential for assessment of the majority of patients who present with nonspecific or vague symptoms and a nondiagnostic ECG because these markers represent one of the two criteria upon which the MI diagnosis rests. Many institutions have initiated Chest Pain Evaluation Centers (CPECs), which are specific protocol-driven treatment areas intended for the systematic and cost-effective care of these high prevalence patients (7). CPECs are usually within or

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3 Nonstandard abbreviations: MI, myocardial infarction; ECG, electrocardiogram; CK-MB, creatine kinase-MB isoenzyme; CPEC, Chest Pain Evaluation Center; CRP, C-reactive protein; cTnT, cardiac troponin T; cTnI, cardiac troponin I; and TIMI, Thrombolysis in Myocardial Infarction.

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The Continuum of Acute Coronary Syndromes

![Diagram of the Continuum of Acute Coronary Syndromes]

Fig. 1. The continuum of acute coronary syndromes, myocardial ischemia, and risk.

near the Emergency Department and have instituted protocols for monitoring this large group of “rule out MI” patients. Diagnosis and care of the suspected acute coronary syndrome patient, particularly those having nondiagnostic ECGs, has been facilitated by establishment of CPECs.

Although there are views both pro (8) and con (9) regarding the role of rapid, or real-time availability of CK-MB and other cardiac markers, data show an association with both reduction in length of hospitalization and overall laboratory costs in institutions producing shorter turnaround times (10). It is of note that in 4–8% of MI patients the diagnosis is missed, and there is high mortality among this group (11–15). In addition, misdiagnosis of MI represents the highest outlay of malpractice dollars among Emergency Medicine physicians (16).

A major purpose of this overview is to present the role that biochemical markers will play in the future for assessing the continuum of myocardial ischemia, which correlates to a spectrum of risk. Also, we will present current strategies for the diagnosis of suspected MI, how biochemical markers may be a harbinger of adverse outcome, and future strategies for identifying the patient’s location on the acute coronary syndrome continuum. Finally, the role of biochemical markers, combined with clinical variables, for the noninvasive assessment of patients to whom thrombolytic therapy has been administered will be discussed.

CK-MB: Current Strategy

CK-MB has shown to be an important tool in the evaluation of acute coronary syndromes. CK-MB is one of three dimeric isoenzymes comprising total CK activity. All cytoplasmic CK is composed of M and/or B subunits that associate to form CK-MM, CK-MB, and CK-BB isoenzymes. CK-MM predominates in striated muscle, both skeletal and myocardial. In patients having considerable myocardial disease, i.e., aortic stenosis, coronary artery disease, or both, the CK-MB isoenzyme comprises approximately 20% of the total CK in this tissue (17), whereas CK-MB comprises only 0–3% of CK in skeletal muscle (18). It is noteworthy that “normal” individuals have a much lower percentage of CK-MB, in the range of 1.1% (17). “Total CK” refers to the cumulative activity of the MM, MB, and BB isoenzymes in patient samples.

Currently, CK-MB must be considered the benchmark of biochemical markers of myocardial injury and, as such, has been the basis for comparison of other markers. Although CK-MB is diagnostically specific for myocardial injury, skeletal muscle has both higher total CK activity per gram of tissue and may have up to 3% CK-MB (17). This potentiates nonspecificity, particularly in patients with concomitant myocardial and skeletal muscle injury.

To confer greater cardiac specificity to CK-MB measurements, a CK-MB “Relative Index” is frequently calculated according the following equation:

\[
CK-MB \text{ Index} = 100\% \left( \frac{CK-MB}{Total \ CK} \right)
\]

Some authors suggest that CK-MB Index values exceeding 2.5% are associated with a myocardial source of the MB isoenzyme (19); however, a recent review shows that myocardium-related CK-MB has been stated by others to be as low as 2% and as high as 5%, depending on the variability of both the numerator and denominator terms in the relative index (20).

The characteristic rise and fall of CK-MB in serial measurements is nearly pathognomonic for diagnosing MI (21). The first rise in CK-MB after MI requires 4–6 h after onset of symptoms. For diagnosis with high sensitivity and specificity, serial sampling over a period of 8–12 h is required. In the CPEC environment, Gibler et al. (22) examined use of CK-MB in a strategy that included sampling at presentation and then at 3, 6, and 9 h later in >1000 low-risk nondiagnostic ECG patients. Although it must be noted that these patients were at low MI risk, this study documented a sensitivity of 100% and specificity of 98.3% for MI diagnosis in this population of chest pain patients (22). CK-MB is also an essential component in assessing re-infarction or infarct extension in patients. Despite this excellent performance, CK-MB is not the ideal marker because its rise requires 8–12 h after the onset of symptoms for use in diagnosis (21), and tissue specificity is an issue.

Harbingers of Myocardial Infarction

Clinical presentation of MI and cardiac ischemia can have a large variety of symptoms, commonly including chest pain or pressure or epigastric burning discomfort, often with radiation to the neck, arms, shoulders or jaw (23–25); less typical presentations including dyspnea, diaphoresis, nausea, and vomiting may accompany these common symptoms or may be the sole manifestation of ischemia (24, 26). Less frequently, ischemic pain may be described as sharp or pleuritic some time before presentation (24, 26). In fact, ~50% of MIs are heralded by intermittent or “staccato” clinical symptoms to hospital presentation (27). This presentation has been termed “prodromal” and
has been documented in the literature for almost 50 years (28). Patients presenting with prodromal symptoms have a more favorable clinical course initially (29). Four mechanisms possibly explain why previous angina leads to a favorable outcome difference (30). First, previous angina may cause the opening of collateral vessels so that, with occlusion of the main vessel, the territory distal to the blockage is partially perfused. Second, angina may produce ischemic preconditioning through an energy-sparing effect that protects the heart from ischemic damage during coronary occlusion (31). Third, treatment of angina patients with aspirin and heparin may serve to abort or minimize the infarction. Finally, there is speculation that the thrombi in prodromal patients may have a different composition, having clots with a smaller proportion of platelet aggregates and more fibrin strands that are less resistant to lysis. In any case, the prodromal presentation is clinically important because patients who have these symptoms have less release of biochemical markers, and therefore less tissue injury, leading to a more favorable outcome compared with patients in whom coronary occlusion is abrupt (27). Educating the public to recognize prodromal symptoms and seek treatment before longer periods of occlusion could be important to decreasing the mortality of the acute coronary syndromes.

**Acute Coronary Syndrome Continuum**

Much of the role of the laboratory has traditionally been associated with biochemical marker testing for diagnosis of acute MI, assessment of re-infarction or infarct extension, and estimating the quantity of infarcted tissue (infarct sizing). Logically, these roles have been clearly correlated with the WHO criteria for MI diagnosis. The future role of the laboratory will be more closely linked to the continuum of the acute coronary syndromes through risk stratification and “therapeutic monitoring” of thrombolytic therapy, platelet inhibition strategies, and perhaps other interventions such as coronary angioplasty.

The representation of cardiac ischemia as binary by the WHO criteria for MI may be viewed as an anachronism because, as illustrated in Fig. 1, the acute coronary syndromes represent a continuous phenomenon. On a more basic level, the continuum of myocardial ischemia also represents the spectrum of ischemic cell injury, ranging from injury that is clearly reversible to extensive necrosis. The events depicted in Fig. 1 also represent a spectrum of risk for an adverse outcome. Patients with stable angina, i.e., reversible ischemia, or myocardial preconditioning are clearly at lower risk for adverse events in both the short term and long term than patients with Q-wave infarction who have infarcted large areas of their myocardium. Identifying where an individual patient’s disease lies in the continuum of acute coronary syndromes has biological implications regarding the reversibility of injury and quantity of ischemic cell injury, as well as the patient’s relative risk for an adverse outcome.

Much of the focus of biochemical markers has included markers of necrosis, for example, CK-MB. However, other markers and substances shown in Table 1 are released or activated before necrosis and may have an important role in identifying risk in the acute coronary syndrome patient. Increased concentrations of the acute phase proteins C-reactive protein (CRP) and serum amyloid A are known to be nonspecific, but may have a role in identifying patients having unstable coronary plaques. Studies have also investigated the use of CRP for predicting unfavorable outcomes and impairment of left ventricular function resulting from acute cardiac necrosis or previous myocardial infarction (32). The increase in acute phase proteins may indicate plaque disruption that causes release of cytokines from activated monocytes and macrophages at the disrupted site (33). Among other systemic effects, cytokines, including interleukin-6, promote hepatic synthesis of the acute phase proteins. In this way, patients having unstable coronary artery disease who are at increased risk have abnormal circulating concentrations of acute phase proteins (34, 35). A possible component of the observed association between acute phase proteins and increased risk is that these proteins may reflect infectious disease to the coronary vessels (36). In any case, aspirin or other nonsteroidal antiinflammatory agents may reduce risk in coronary artery disease patients, presumably by inhibiting the inflammatory process (32).

Platelet activation is important in the mechanism of thrombus formation and the mechanism of acute coronary syndromes. Indicators of platelet activation such as platelet function assays or P-selectin may help assess a patient’s tendency for intracoronary thrombosis. Platelet activation can result from contact with exposed collagen, thrombin, and/or other agonists induced by plaque disruption. P-selectin is an adhesion molecule expressed on the surface of activated platelets (37). Expression of this protein is increased on the surface of platelets in patients with symptomatic coronary artery disease (37). Thus P-selectin may be a marker of “angry” platelets that indicates their tendency to adhere to leukocytes, causing their accumulation and consequent thrombotic complications in the ischemic myocardium (37).

Thrombus formation is fundamental to blockage of the infarct-related artery; therefore, markers of thrombosis, including soluble fibrin and fibrin degradation products, may also reveal a recent thrombotic process or risk of an impending event. These markers are characteristics of the procoagulant fibrinolytic activity. Although not sensitive enough to diagnose MI, insoluble fibrin and cross-linked fibrin degradation are increased in patients who are at higher risk for complications (38). Physiologically, these markers are thought to indicate increased fibrinolysis before development of MI (38).

A marker that is indicative of myocardial ischemia occurring before frank necrosis would be helpful for locating a patient’s position on the acute coronary syndrome continuum. Although there is no definitive information, glycogen phosphorylase-BB isoenzyme is in-
creased upon ischemia without necrosis and may represent a marker of ischemia rather than necrosis (39).

Glycogen phosphorylase-BB release is thought to be linked to the sudden burst of glycogenolysis that occurs in the injured myocardium after acute MI. Taken together, the markers of plaque rupture (CRP and serum amyloid A), indicators of intracoronary thrombosis (P-selectin and soluble fibrin), myocardial ischemia (glycogen phosphorylase-BB), and markers of necrosis could be combined with clinical indicators, the ECG, echocardiogram, and imaging studies to form an integrated combined model for optimum assessment of patient risk.

**Table 1. Biochemical markers in the continuum of acute coronary syndromes.**

<table>
<thead>
<tr>
<th>Pathophysiology</th>
<th>Biochemical marker</th>
<th>Molecular mass, Da or g/mol</th>
<th>Cardiac-specific</th>
<th>Type of assay</th>
<th>Duration of increase</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque rupture</td>
<td>CRP</td>
<td>120 000</td>
<td>NA^a</td>
<td>Latex photometric immunoassay or rate nephelometry</td>
<td>48–72 h</td>
<td>CRP and serum amyloid A are acute phase proteins; may indicate plaque disruption and be prognostic in unstable angina patients</td>
</tr>
<tr>
<td>Plaque rupture</td>
<td>Serum amyloid A</td>
<td>12 500</td>
<td>NA</td>
<td>Sandwich-type enzyme immunoassay</td>
<td>48–72 h</td>
<td></td>
</tr>
<tr>
<td>Intracoronary thrombosis</td>
<td>Platelet activation</td>
<td>NA</td>
<td>No</td>
<td>Functional assays using platelet agonists</td>
<td></td>
<td>Must be performed soon after blood collection</td>
</tr>
<tr>
<td>Intracoronary thrombosis</td>
<td>P-selectin</td>
<td>140 000</td>
<td>NA</td>
<td>Flow cytometric assay</td>
<td></td>
<td>Marker of angry platelets; may indicate risk for acute coronary events</td>
</tr>
<tr>
<td>Soluble fibrin</td>
<td></td>
<td></td>
<td></td>
<td>ELISA</td>
<td></td>
<td>Marker of procoagulant fibrinolytic activity; may predict patients at higher risk for MI-related complications</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Glycogen phosphorylase-BB</td>
<td>177 000</td>
<td>+++b</td>
<td>Immunoenzymometric assay</td>
<td>8 h</td>
<td>Marker of cardiac ischemia</td>
</tr>
<tr>
<td>Myocardial necrosis^c</td>
<td>Myoglobin</td>
<td>18 000</td>
<td>No</td>
<td>Immunoassay</td>
<td>12–24 h</td>
<td>Markers of cardiac necrosis</td>
</tr>
<tr>
<td>Myocardial necrosis^c</td>
<td>CK-MB, mass assays</td>
<td>85 000</td>
<td>++</td>
<td>Immunoassay</td>
<td>24–36 h</td>
<td></td>
</tr>
<tr>
<td>Myocardial necrosis^c</td>
<td>cTnT</td>
<td>37 000</td>
<td>++++</td>
<td>Immunoassay</td>
<td>10–14 days</td>
<td></td>
</tr>
<tr>
<td>Myocardial necrosis^c</td>
<td>cTnl</td>
<td>23 500</td>
<td>++++</td>
<td>Immunoassay</td>
<td>4–7 days</td>
<td></td>
</tr>
</tbody>
</table>

^a NA, not applicable.

^b ++, ++++, and ++++ indicate degrees of cardiac specificity.

^c Time of first increase for the markers are 1–3 h for myoglobin, 3–4 h for CK-MB mass, 3–4 h for cTnT, and 4–6 h for cTnl.

Increased upon ischemia without necrosis and may represent a marker of ischemia rather than necrosis (39). Glycogen phosphorylase-BB release is thought to be linked to the sudden burst of glycogenolysis that occurs in the injured myocardium after acute MI. Taken together, the markers of plaque rupture (CRP and serum amyloid A), indicators of intracoronary thrombosis (P-selectin and soluble fibrin), myocardial ischemia (glycogen phosphorylase-BB), and markers of necrosis could be combined with clinical indicators, the ECG, echocardiogram, and imaging studies to form an integrated combined model for optimum assessment of patient risk.

**Risk Stratification of Acute Coronary Syndrome Patients**

Much interest has been generated by outcome-based studies indicating that biochemical markers are useful for stratifying risk in acute coronary syndrome patients. The main focus of this interest has been on CK-MB, cardiac troponin T (cTnT), and cardiac troponin I (cTnl).

cTnT and cTnl represent a new generation of biochemical markers that may provide an additional clinical tool for assessment of the acute coronary syndromes. Along with troponin C, troponin T and troponin I are essential components of the contractile complex of both skeletal and myocardial striated muscle (40, 41). Troponin T functions to bind the troponin complex to the tropomyosin strand; troponin I functions to inhibit the activity of actomyosin ATPase; and troponin C serves to bind four calcium ions, thus regulating contraction. Clinical interest in the proteins of the troponin complex is driven by cardiac-specific isoforms of troponin T and troponin I that have been purified, allowing antibody production and development of immunoassays that are virtually cardiac-specific (42, 43). The amino acid sequence for troponin C is identical in cardiac and skeletal muscle tissue, precluding use of this protein as a specific cardiac marker (40).

The release and clearance mechanisms of troponin T and troponin I are incompletely understood. Although both troponin T and troponin I are structural proteins, early reports suggested that a “cytosolic” pool of these proteins was released into circulation after cell injury. This cytosolic pool for cTnT has been reported at 6–8% (44), whereas the soluble cTnl pool is reportedly 2.8% (45). More recently, reports indicate that cTnl is released as troponin T/troponin I/troponin C or troponin I/troponin C complexes (46); cTnT may have a different release sequence. The different possible release forms of cTnl and cTnT underscore the need for standardization and comprehensive characterization of assays for these proteins. In addition, issues regarding release emphasize the need to consider cTnT and cTnl as different proteins because they differ in biological function, molecular weight, re-
lease mechanism, and other important characteristics that may impact their clinical utilization.

Currently there is one quantitative and one qualitative assay for cTnT cleared by the US Food and Drug Administration; both of these assays use the same antibody pairs and results correlate well. cTnT increases have been documented in patients with end-stage renal disease (44); however, the clinical meaning of these increases is unclear pending completion of properly designed outcome studies.

Currently there are numerous cTnI qualitative or quantitative immunoassays that have demonstrated excellent potential for clinical use in the diagnosis of MI and that have been cleared by the US Food and Drug Administration. cTnI may have an important role in real-time strategies for evaluating acute coronary syndrome patients, an area that has been of intense interest, discussion, and study over recent years (47,48). Data are rather limited, but indicate that cTnI is a specific marker in cases involving skeletal muscle injury and renal failure (49).

Over the past few years, a number of clinical outcome-based studies have demonstrated that patients with acute cardiac ischemia in whom CK-MB, cTnI, and/or cTnT was increased are at increased risk for adverse events including myocardial infarction or cardiac death (50,51). These data beg a particularly important question in this age of increased cost-containment: which assay or assays should be performed for utilization in risk stratification? Review and metaanalysis of this question is confounded by the many different methods utilized for CK-MB measurement (50) and also for cTnI. The fact that different methods for these markers have different analytical sensitivities, susceptibility to interference, and performance characteristics may have important implications on which assays provide useful information.

A study designed to compare the usefulness of CK-MB, measured by a state-of-the-art mass assay, cTnT, and the ECG for risk assessment was performed as a substudy of the GUSTO IIa trial (52). This GUSTO IIa substudy included 854 patients, all of whom had symptoms of cardiac ischemia within 12 h of enrollment and an abnormal ECG. GUSTO IIa showed that the higher the cTnT concentration at presentation, the greater the risk of 30-day mortality (52). Also, patients that tested positive for cTnT had a threefold increase in morbidity compared with patients who tested negative. Furthermore, Table 2 shows that cTnT was the most powerful predictor of death in the 30 days after clinical presentation in a logistic regression model developed from GUSTO IIa data. The combined analysis in Table 2 shows that among the ECG, cTnT, and CK-MB, cTnT added the most information regarding risk of 30-day mortality, and CK-MB provided no added value beyond that provided by the ECG and cTnT (52).

A separate investigation, termed the FRISC study, examined peak cTnT over the 24-h period after initial presentation in 976 patients, all of whom had unstable coronary artery disease (53), and correlated cTnT concentration with outcome measures that included cardiac death and MI over the following 150 days. A key finding of the FRISC study was that risk of an adverse cardiac outcome increased as the cTnT value increased, as indicated in Table 3. This study concluded that cTnT measurement in the first 24 h provided valuable prognostic information over the following 5 months, which was independent of age, hypertension, number of antianginal drugs, and ECG changes (53).

An extension of the FRISC study examined whether cTnT concentrations could be useful for identifying patients with unstable coronary artery disease who might benefit from therapeutic intervention. This issue was investigated by measuring cTnT concentrations in serum from 971 patients who received either a placebo or low molecular weight heparin in short-term (6-day) or long-term (5-week) regimens (54). Among patients with cTnT <0.1 µg/L, short-term treatment showed a trend toward significance in that the incidence of death and/or MI was reduced from 2.4% to 0% (P = 0.12). In patients with cTnT ≥0.1 µg/L, the incidence of death and/or MI was 6.0% and 2.5% in the placebo and short-term treatment groups, respectively (P <0.05). In the patients who received long-term treatment with low molecular weight heparin, patients with cTnT ≥0.1 µg/L died and/or experienced MI at a rate nearly double that of the placebo group (14.2% vs 7.4%; P <0.01). On the other hand, cTnT concentrations <0.1 µg/L identified a low-risk group in whom death and MI showed no difference between the treated and placebo groups. Increased cTnT concentrations clearly identified patients who would benefit from long-term treatment with low molecular weight heparin (54).

cTnI was investigated in a substudy of the TIMI IIIb for risk stratification of acute coronary syndrome patients, using an endpoint of 42-day mortality (47). In this study,
cTnI was compared with CK-MB mass in 1404 unstable angina or non-Q-wave infarction patients. cTnI concentrations ≥0.4 μg/L were associated with significantly higher mortality at 42 days than lower concentrations among patients with unstable angina or non-Q-wave infarction and was an independent predictor of short-term mortality after adjustment for age ≥65 years and the presence of ST-segment depression. cTnI ≥0.4 μg/L appeared to indicate increased risk of mortality (risk ratio, 3.1) even in patients whose CK-MB measurements were not abnormally increased. This study concluded that cTnI provides for the early identification of patients at increased risk of death by retrospective measurement of cTnI (47).

A logical question in the context of risk stratification was: Do we need to measure both cTnT and cTnI? To directly compare cTnT and cTnI, an extension of GUSTO IIa was performed in 755 patients (55). Although 90% of the results were concordant using positive/negative cutoffs from the package insert from each respective assay, a significantly greater number of patients were cTnT-positive but cTnI-negative than who were cTnT-negative but cTnI-positive (P <0.001). cTnT measurements in specimens collected at enrollment appeared to be more useful than cTnI for predicting 30-day mortality (55).

Because cTnT and cTnI cutoffs were used in examining these data, different decision points may have yielded different results for predicting 30-day mortality. For this reason, ROC curves were plotted for cTnT and cTnI because this strategy evaluates the relative performance of assays independent of cutoff (56). Using 30-day mortality as the outcome, the area of the ROC curve for cTnT was significantly larger at 0.68 than that for cTnI at 0.64 (P = 0.002). These data indicate that the cTnT was a more useful test for predicting 30-day mortality in the GUSTO IIa population (55).

Furthermore, Table 4 shows results of logistic regression modeling used to examine cTnT, cTnI, and the ECG as predictive variables alone or in combination. Considering each of the variables individually, cTnI was most useful for predicting 30-day mortality (Table 4). When cTnI and the ECG were put in the logistic regression model first, cTnT added significant information (P = 0.045). However, when cTnI was the added variable to a cTnT and ECG model, there was no significant increase in the ability to predict 30-day mortality (Table 4). Although cTnT provided the most information regarding prediction of 30-day mortality (55), the characteristics of either the cTnT or cTnI results may be method-dependent, as are those for CK-MB (57). Thus, use of different or more sensitive cTnT or cTnI assays may indicate different results. Although the GUSTO IIa population included was large, only ~10% (n = 74) of the patients showed discordant cTnT and cTnI results.

Elucidating the nature and complexity of cTnI release and metabolism is an area of active investigation. Other immunoassays for either cTnT or cTnI may show different properties, depending on the minimum detectable concentration of the cTnI assay used and whether the assay detects the cTnT or cTnI released in the free or complex form, oxidized or reduced, phosphorylated or not, and other factors that may affect the target epitope, antibody properties, and/or other assay conditions. A recent manuscript characterizes the reactivity of several commercial assays for different forms of cTnI (58). This area of investigation may yield information that has major impact on the clinical utilization of cTnI.

Hamm et al. (59) investigated the use of cTnI and cTnT in chest pain patients having a nondiagnostic ECG, using qualitative assays for these markers. This study showed clearly that these qualitative assays were useful for predicting risk in a nondiagnostic ECG, CPEC-like population.

When interpreting the results of studies using qualitative devices, one must be cognizant of the cutoff concentration of the device, i.e., the lowest marker concentration that produces a positive result. A convenient benchmark for evaluating the cutoffs of these devices is by examining the corresponding quantitative concentration of the marker defined for diagnosis of MI by WHO criteria, derived from ROC analysis. Recall that one of the WHO criteria involves documenting the temporal rise in cardiac markers, i.e., proteins (cTnT or cTnI), or measurement of enzymes by either functional activity or as a protein (CK-MB). In the study by Hamm et al. (59), the cutoff for the cTnI device was twofold higher than the cutoff recommended for diagnosis of MI according to WHO criteria. On the other hand, the cutoff for the cTnI device was less than that for WHO criteria MI (59). In fact, the cTnI device used in the study by Hamm et al. is no longer available but has been replaced by a device having a lower cutoff.

### Table 4. Relative value of cTnT, cTnI, and the ECG in the prediction of 30-day mortality.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate</td>
<td>cTnT</td>
<td>21.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Univariate</td>
<td>ECG</td>
<td>14.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Univariate</td>
<td>cTnI</td>
<td>12.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Multivariable</td>
<td>Added variable</td>
<td>Additional χ²</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>cTnT</td>
<td>8.03</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>ECG</td>
<td>9.96</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>cTnI</td>
<td>0.84</td>
<td>0.675</td>
</tr>
</tbody>
</table>

**Assessment of Reperfusion after Thrombolytic Therapy**

Besides stratification of risk in acute coronary syndromes, biochemical markers will also have an important role in monitoring the success of thrombolytic therapy. This role will be driven because the open artery hypothesis has been proven by improved outcome (60, 61) and increased left ventricular function (62) as a result of reestablishment of patency in the infarct-related artery after MI. In accordance with convention, patency in the infarct-related artery is graded by angiography according to the TIMI criteria, in which
TIMI 0 is no perfusion past the occlusion; TIMI 1 is penetration past the occlusion without perfusion; TIMI 2 is partial perfusion past the occlusion; and TIMI 3 is complete perfusion (63). The objective of noninvasive assessment of reperfusion is rapid identification of the 20–25% of patients in whom the occlusion persists (TIMI 0 or 1 flow) in the 90–120 min after thrombolytic therapy. Other methods for assessing patency include coronary angiography; although considered the gold standard, this method is associated with high cost, limited availability, and increased morbidity when performed acutely (64, 65). Clinical indicators such as detection of reperfusion arrhythmia and cessation of pain are unreliable indicators of patency (66). A strategy that includes biochemical markers to monitor the “wash out” phenomenon (67) when (if) patency is reestablished in the infarct-related artery could be a valuable contribution.

Use of various biochemical markers, including CK-MB (68, 69), the MM and MB subtypes (70, 71), troponin T (72), and troponin I (73), has been investigated for noninvasive assessment of reperfusion; however, none of these markers has a low molecular weight or shows the early release characteristics of myoglobin, which is washed out rapidly after coronary reperfusion (74). Largely because of these characteristics, myoglobin has been examined for noninvasive assessment of myocardial perfusion after thrombolytic therapy (75). Other clinical variables can provide information regarding successful reperfusion but are unreliable by themselves (76). Therefore, a model that combines clinical variables and the optimum strategy for CK-MB and myoglobin measurement can provide clinicians a tool for assessing how likely successful reperfusion occurred in an individual patient.

Such a combined model was recently developed (77) using data collected from 96 patients enrolled in the Thrombolysis and Myocardial Infarction-7 study (78). All patients in the Thrombolysis and Myocardial Infarction-7 study received thrombolytic therapy and then coronary angiography ~2 h later to grade coronary blood flow; coronary artery patency was graded according to TIMI classification (63). In this study, two classifications of TIMI flow were considered successful reperfusion. The first included the original classification of TIMI 2 or 3 for successful reperfusion, with TIMI 0 or 1 grade flow considered failed thrombolysis. The second classification was based on recent outcome-based data, and considered TIMI 3 only as successful reperfusion, with TIMI 0, 1, and 2 grade flow as failed thrombolysis (79).

Blood specimens for measurement of the biochemical markers were collected before starting thrombolytic therapy, and then 30, 90, and 180 min after thrombolytic therapy. All 96 patients had a “near-catheterization” specimen collected within 10 min of the angiographic study. Myoglobin was measured in all samples, using the two-site immunoassay method available on the Stratus II (Dade Diagnostics); CK-MB measurements were performed with the ICON CK-MB kit (Hybritech Inc.). Logistic regression modeling was used to combine the biochemical markers and clinical variables. The output of the logistic regression modeling is always a number between zero and unity; this result will be referred to as “model output” in subsequent discussion and is the y-axis in Fig. 2.

Preliminary models were developed that included various myoglobin strategies alone, selected clinical variables, and myoglobin and clinical variables combined; various CK-MB models had been analyzed previously (68, 69). The optimum model included a single myoglobin

![Fig. 2. Distribution of data (left panel) and the resulting ROC curve (right panel) for use of a strategy combining a single myoglobin value, CK-MB slope, and clinical variables for discriminating TIMI 0–1 from TIMI 2–3 coronary flow. Model Output on the y-axis of the left panel represents the results of logistic regression modeling (always a number between zero and unity) for combining the biochemical markers and clinical variables. The area of the ROC curve (right panel) was 0.88. Example A in the left panel represents placing a cutoff at a model output of 0.4; this cutoff corresponds to ROC position A in the right panel, having sensitivity of 48% and specificity of 82%. Example B in the left panel represents placing a cutoff at a model output of 0.24; this cutoff corresponds to ROC position B in the right panel, having sensitivity of 83% and specificity of 78%. Example C in the left panel represents placing a cutoff at a model output of 0.10; this cutoff corresponds to ROC position C in the right panel, having sensitivity of 96% and specificity of 55%.](https://example.com/fig2.png)
measured at catheterization, the slope of CK-MB release, time from chest pain to start of thrombolytic therapy, and chest pain grade (from 0 to 10, with 0 being no pain) at the time of cardiac catheterization (77). Myoglobin added significantly to the ability of the model to predict TIMI 0–1 coronary flow, i.e., failed reperfusion vs successful reperfusion grade TIMI 2–3 flow (P <0.044). Fig. 2 (left panel) displays a plot for the ability of the model to separate TIMI grade 0–1 coronary flow from that of TIMI 2–3 flow; Fig. 2 (right panel) shows the associated ROC curve for the model with three points shown as an example; the area of this ROC curve was 0.88. For predicting TIMI 3 grade flow vs grades 0–2, the model remained highly significant (P <0.0043) and showed an ROC curve area of 0.74.

Although combining near catheterization myoglobin, CK-MB slope, and clinical variables yielded a high ROC area, the strategy will not accurately predict reperfusion status in all patients. This may be unavoidable for at least three physiologically based reasons. First, strategies that use biochemical markers are based on differences in the washout phenomenon that occurs after patency has been reestablished (67). The washout model often used to characterize biochemical markers showing promise for the assessment of patency is acute angioplasty. However, this model may not rigorously simulate the washout phenomenon that occurs after thrombolytic therapy, because angioplasty restores patency abruptly, producing dramatic increases in the biochemical markers (80). In contrast, the restoration of patency after thrombolytic therapy is a more dynamic process in which many patients have repeated opening and closing of the infarct-related artery in a stuttering pattern. Intermittent patency is probably caused by alterations in coagulation factors, platelet function, or other potentiating factors that affect procoagulant activity and contractility of coronary arterial muscle, which could blunt the washout of biochemical markers. Second, individual patient variables such as extent of infarction, collateral flow to the infarcted area, tissue stunning, and/or hibernation as well as blood pressure, may influence noninvasive strategies for assessing patency. A third issue involves the use of angiography to adjudicate patency. Although it is the gold standard for evaluating coronary patency, angiography represents a “snapshot” of coronary patency; this technique is not a definitive indicator because it cannot measure how long the documented perfusion status has existed in the infarct-related artery either before or after imaging, which would influence washout. Thus, some discrepancy must be expected because of the dynamic physiological nature of patency restoration after thrombolytic therapy and the uncertainties in angiographic measurement.

This study shows that a model consisting of biochemical markers, i.e., a single myoglobin obtained between 60 and 150 min and the slope of CK-MB release after starting thrombolytic therapy combined with time from the onset of symptoms to thrombolytic therapy and the chest pain grade, may provide important clinical information for patients (77).

**Conclusions**

The acute coronary syndromes represent a continuum of myocardial ischemia ranging from angina, which indicates reversible tissue injury, through frank MI with extensive tissue necrosis. A new generation of biochemical markers for indicating plaque disruption, platelet reactivity, early myocardial necrosis, and effectiveness of thrombolytic therapy offer promise for better assessment of patient risk so that clinicians may intervene to avoid adverse outcomes. Although historically CK-MB has proven a most useful marker for the diagnosis of MI according to WHO criteria, cTnT and cTnI have emerged as sensitive, more cardiac-specific clinical indicators that are useful for MI diagnosis and, importantly, for stratification of risk. Because of a paucity of data, however, CK-MB must be considered an important indicator for assessment of re-infarction or infarct extension. Other biochemical markers, including the acute phase reactants CRP and serum amyloid A, and markers of platelet reactivity, including P-selectin, as well as markers of thrombosis may become useful for identifying a patient’s location on the spectrum of acute coronary syndrome and therefore the risk of adverse events. For assessing reperfusion after thrombolytic therapy, a strategy that includes myoglobin, CK-MB, and clinical indicators, including time to treatment and the chest pain grade, show high efficiency and may provide an important new tool to clinicians. Biochemical markers will continue to play a traditional role in the workup of MI patients, but they are also developing into an important adjunct for patient decision-making along with the EGG and, most importantly, clinical judgment.

R.H.C. is or has been a consultant for and/or owns stock in Abbott Laboratories, Boehringer Mannheim Corp., Dade Behring, Inc., and First Medical, Inc., and holds an international patent for a method of noninvasive assessment of reperfusion.

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