Review Article

Current Trends in Diagnostic Biomarkers of Acute Coronary Syndrome

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Abstract

The diagnosis and management of patients with acute coronary syndrome (ACS) have evolved dramatically over the past decade. Biomarkers play an important role in the diagnosis of ACS, especially in unstable angina and non-ST-segment elevation myocardial infarction. Among these, cardiac troponin and creatine kinase appear to be the most sensitive and specific markers of myocardial injury. Recent studies have revealed several novel biomarkers. Elevated levels of C-reactive protein and interleukin-6 are strong independent markers of increased mortality among patients with ACS. However, the ideal biomarkers that offer early detection, risk stratification, selection of therapy, monitoring disease progression, and treatment efficacy remain to be elucidated. This review assesses limitations and contemporary needs for biomarkers in the context of diagnosis of ACS. It also discusses the newly developing technologies for novel biomarkers or novel biomarker protein signatures discovery, and importance of point-of-care testing for future management.

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Introduction

Advances in our understanding of the pathophysiology of acute coronary syndrome (ACS) have led to the marked increase in development of biomarkers for diagnosis, risk stratification, therapeutic decision-making, and assessment of clinical outcomes. Patients with ACS are subdivided into the following 2 major categories based on the 12-lead electrocardiogram (ECG): those with new ST-elevation on the ECG that is diagnostic of acute ST-elevation myocardial infarction (STEMI) and those who present with ST-segment depression, T-wave changes, or no ECG abnormalities [non-ST elevation ACS (NSTEACS)]. The latter encompasses both unstable angina (UA) and non-ST-elevation myocardial infarction (NSTEMI).[1,2] This group comprises a growing number of patients with ACS and is emerging as a major public health problem worldwide, especially in Western countries, Asia and other developing countries.[3-5]

In the majority of patients presenting with ACS, thrombus is partially obstructive or only transiently occlusive, and results in coronary ischaemia without persistent ST-segment elevation (UA or NSTEMI). In the remaining patients with ACS, the intracoronary thrombus completely occludes the culprit vessel, resulting in STEMI. Clinicians must make a critical decision for the diagnosis and management of ACS, based on the patient history, physical examination, 12-lead ECG, cardiac biomarkers results, and non-invasive risk stratification. The ECG is the most readily available tool for identifying patients with STEMI who are likely to have myocardial infarction (MI). Establishing the correct diagnosis in patients without ST-segment elevation, however, can be more challenging. The ECG is often non-diagnostic for acute chest pain, and in fact, the sensitivity of the baseline ECG for detecting acute myocardial infarction (AMI) is only 60%.[6] The physical examination can also be inadequate; specifically, atypical chest pain is often difficult to distinguish from chest pain of cardiac origin, and up to 33% of patients with ACS have no chest pain.[7]

These imperfect strategies result in costly and inappropriate management decisions. Approximately half of patients with acute chest pain who have initial diagnostic findings of ACS and are admitted to the hospital are later found not to suffer from ACS.[8] Misdiagnosis has been reported to be the main cause of treatment delays.[9] On the other hand, undetected infarctions remain a serious public health issue and represent the leading cause of malpractice cases in the emergency setting,[10] thus leading to cardiac biomarkers as an integral component in the care of ACS patients, specifically for UA and NSTEMI. Along

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with the efforts in evaluating patients with ACS, a large number of putative cardiac biomarker studies have been performed for the diagnosis of ACS. As a result, a panel of biomarkers has been recommended for routine use in the care of ACS patients.[1] This review assesses the limitations and contemporary needs for diagnostic biomarkers in ACS, as well as the challenges in the development of new technologies to integrate biomarkers into clinical practice.

Current Practice of Diagnostic Biomarkers in ACS

Cardiac Troponin

Several well-designed studies have shown that cardiac troponin (cTnI and cTnT) to be the most diagnostically sensitive and specific biomarker of myocardial injury.[11-13] Moreover, on the basis of improved sensitivity and superior tissue specificity compared to other biomarkers of necrosis, cTn is recommended for the diagnosis of AMI by the National Academy of Clinical Biochemistry (NACB) Laboratory Medicine Practice Guidelines[1] and the International Committee of Experts in Epidemiology, Pathology, Clinical, and Laboratory Medicine.[14] The kinetics of cTnI and cTnT are similar; cTnI and cTnT are detectable in the serum within 4 to 12 hours after the onset of AMI, and depending on the duration of ischaemia and reperfusion status, peak values occur 12 to 48 hours from symptom onset (Fig. 1).[15] Therefore, serial sampling, including a baseline sample and follow-up examination 8 to 12 hours after symptom onset is recommended.[16] Because of the tissue specificity of cTn, any reliably-detected concentration of cTn in the peripheral circulation as abnormal and indicative of myocardial injury.[17] Moreover, several studies showed that patients presenting with an increased cTn concentration had a poor prognosis compared to those without detectable cTn.[18] Although cTnI is cleared more quickly from the circulation than cTnT, both isoforms remain elevated in the serum for several days after injury, allowing for diagnostic confirmation, even in patients presenting with delayed symptoms. Because of the long half-lives, one of the disadvantages of using cTn is that neither cTnI nor cTnT assays can be used for detection of re-infarction after an index event. The other disadvantage is that cTnT is present in small amounts in skeletal muscle and is re-expressed in diseases that involve skeletal muscle degeneration (e.g., Duchenne muscular dystrophy).[19] Moreover, an elevated cTn without clinical evidence of ACS should prompt a search for other possible myocardial injuries, including cardiac trauma, congestive heart failure, and hypertension, as an elevated cTn does not indicate its mechanism.[6]

Creatine Kinase

When the cTn is not available, the best alternative marker is the creatine kinase-myocardial band (CK-MB), which is measured by mass assay.[1,14] In the early days, the elevated serum levels of CK-MB, the cardiac-specific isoform of CK, was used for the diagnosis of myocardial necrosis. This measure satisfied one component of the diagnostic criteria for MI, as proposed by the World Health Organization, and was later extended for monitoring trends and determinants in a cardiovascular disease study.[14,20] CK-MB is predominantly found in the myocardium. Elevation of CK-MB occurs 4 to 6 hours after the onset of AMI and remains for 24 to 48 hours (Fig. 1).[21] Similar to cTn, serial testing of CK-MB has been suggested to increase the sensitivity and specificity in detecting myocardial injury.[22] Although some studies have suggested serial measurement of CK-MB to qualitatively estimate the infarct size, many studies have revealed that a single measurement of plasma cTn can be used as a convenient, cost-effective, and non-invasive method.[23,24] CK-MB is relatively sensitive, but the specificity is in doubt as CK-MB is elevated in any condition following acute or chronic muscle injury, or in patients undergoing a surgical procedure.[25] Furthermore, CK is present in skeletal muscle, intestine, diaphragm, uterus, and prostate, and thus the specificity of CK-MB is impaired in the setting of injury to these organs.[26] In the foreseeable future, CK-MB testing will largely be replaced by cTn as the gold standard.

Myoglobin

The NACB Laboratory Medicine Practice Guidelines have recommended myoglobin in addition to cTn for the diagnosis of AMI patients who present within 6 hours of onset of symptoms.[1] The serum myoglobin level rises faster than CK-MB and cTn, reaching twice normal values with 2 hours and peaking within 4 hours of AMI symptom onset (Fig. 1). Myoglobin achieves its maximal diagnostic sensitivity within 5 hours of symptom onset.[27] The main

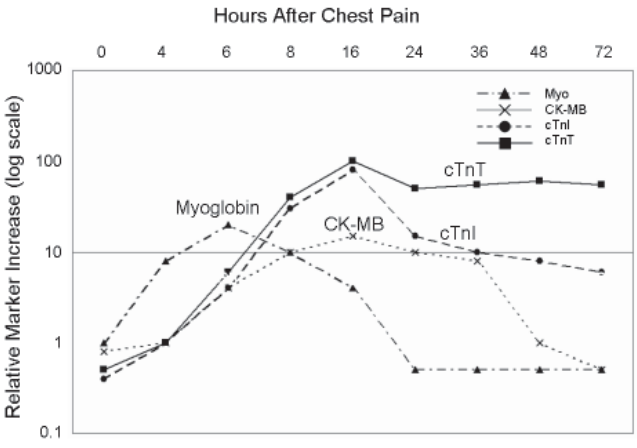

Fig. 1. Temporal release of myoglobin, CK-MB, cTnI and cTnT. (Reproduced with permission from: Christenson RH, Azzazy HME. Biomarkers of necrosis: past, present and future. In: Marrow DA, editor. Cardiovascular Biomarkers: Pathophysiology and Clinical Management. New York: Humana Press, 2006.)

advantage of myoglobin is early detection of patients with AMI. Some studies have suggested that the combined use of myoglobin and a more specific marker of myocardial necrosis (cTn or CK-MB) would be useful for early and rapid recognition of AMI.[28,29] The disadvantage of using myoglobin alone is that it has poor specificity for AMI in patients with concurrent trauma or renal failure.

Natriuretic Peptides

B-type natriuretic peptide (BNP) and its prohormone, N-terminal pro-BNP (NT-proBNP), are neurohormones secreted from cardiac ventricles in response to ventricular wall stress.[30] BNP, an established biomarker for patients with heart failure, and NT-proBNP are elevated in patients with ACS and can identify ACS patients who are at higher risk for adverse cardiovascular events, including heart failure and death.[31-33] The utility of BNP and NT-proBNP as diagnostic biomarkers for ACS is based on the premise that increases in the diameter of and pressure within the left ventricle during remodelling after a transmural infarction, or as a consequence of prior ischaemic damage.[1] However, similar to the above-mentioned biomarkers, these peptides have poor specificity for the diagnosis of ACS since elevated levels can be found in patients with renal failure, primary aldosteronism, congestive heart failure, and thyroid disease.[21,34]

Emerging Diagnostic Markers of ACS

Some cardiac biomarkers do not require myocardial cell death for release, and are thus referred to as myocardial ischaemia biomarkers. These include biomarkers of inflammation and platelet activation. As the prototypical acute phase reactants, C-reactive protein (CRP) and interleukin-6 (IL-6) have been the focus of investigations for the diagnosis of ACS. The CRP level certainly rises as a consequence of the inflammatory response to myocardial necrosis.[35] Some studies have reported significant correlations between CRP concentrations and the size and extent of necrosis.[36] Several primary prevention studies have demonstrated that the baseline CRP concentration can predict cardiovascular events.[37,38] A landmark study by Liuzzo et al[39] showed that patients presenting with UA who had elevated levels of CRP and serum amyloid A had a higher rate of death, AMI, and the need for revascularisation compared with patients without elevated levels. The Fragmin and Fast Revascularisation During Instability in Coronary Artery Disease II trial (FRISC) study group showed that the circulating level of IL-6 is a strong independent marker of increased mortality among patients with UA and is useful in directing subsequent care.[40] However, the best timing for measurement of CRP and IL-6 for diagnosis and risk stratification of ACS remains uncertain. Moreover, the Centers for Disease Control and Prevention and the American Heart Association (AHA) published a scientific statement on the use of CRP and other inflammatory markers, and advised that CRP might be useful to address and tailor intensive medical treatment after ACS, but no definite evidence existed to support such a strategy.[41] More recently, the NACB Laboratory Medicine Practice Guidelines have suggested that CRP should not be considered in the initial evaluation and assigned CRP a class IIA (level of evidence A) recommendation for early risk stratification in ACS in addition to cTn, while the use of CRP as a sole marker is discouraged.[1]

The other inflammatory biomarkers under investigation for the diagnosis and prognosis of ACS are monocyte chemoattractant protein-1 (MCP-1), IL-18, and IL-10.[42] The MCP-1 plasma concentration is associated with different cardiovascular risk factors, and a greater risk of developing a cardiovascular event in the future.[43,44] A prospective study has reported that the serum IL-18 level is a strong independent predictor of death from cardiovascular causes in patients with coronary artery disease.[45] IL-10 and other inflammatory biomarkers, including macrophage inhibitory cytokine-1 and soluble TNF receptors (sTNFR1/sTNFR2), have shown promise in the prediction of the incidence of coronary artery disease, but these makers require further study for possible application in ACS risk stratification.[42]

Finally, there are some new groups of biomarkers that may become useful for the diagnosis of ACS. Most of these biomarkers are still under intense investigation. Most of the prospective studies have demonstrated that these markers were of prognostic value for ACS. However, the diagnostic significance of these markers remains to be elucidated.

(i) Biomarkers of Oxidative Stress

Elevated levels of lipoprotein-associated phospholipase A-2, secretory phospholipase A2, oxidised low-density lipoprotein, and myeloperoxidase are reported to have a significant association with the risk of death or MI in ACS patients.[46]

(ii) Biomarkers of Tissue Necrosis

Fatty acid binding proteins (FABPs), free fatty acid unbound to albumin (FFAu), and ischaemia modified albumin (IMA) appear to play an important role in the diagnosis of ACS. Elevated levels of heart-type FABP has been demonstrated soon after the onset of infarction; heart-type FABP has been proposed to be an early marker of ACS.[47] FFAu is increased in 100% of MI patients on admission, whereas only 22% of these patients have increased cTn at presentation, indicative of the early appearance of this marker in the circulation before traditional markers.[48] A recent study showed the sensitivity and sensitivity of IMA for myocardial necrosis were 88% and 94%, respectively.[49] However, many questions remained unanswered. Although

these markers may have an important role in certain clinical settings in the future, their precise role is currently unknown.

Limitations of Diagnostic Biomarkers in ACS

Each of the markers discussed in this review has limitations. An ideal marker is one in which there is a specific easily measurable increase that clearly aligns with a diagnosis or a predictable outcome. cTn appears to be the most sensitive and specific biomarker among all other diagnostic biomarkers for ACS. However, there is a consensus that a single measurement of cTn on arrival of the patient to the hospital is insufficient because a single measurement of cTn will not detect 10% to 15% of at-risk patients.[50]

The optimal timing for obtaining a sample for measurement of biomarkers is another limitation. Cardiac troponin and CK-MB levels rise within 3 to 4 hours and myoglobin rises 1 to 2 hours after the onset of symptoms. Although cTn and CK-MB appear to be sensitive, the temporal rise in the serum concentrations of these biomarkers does not permit the early detection of myocardial necrosis (1 to 3 hours)[1] and does not support maximal sensitivity of these markers until 6 hours after the onset of symptoms.[51]

Most importantly, the rapid and correct diagnosis of ACS, specifically UA or NSTEMI, is a challenging and demanding issue. Point-of-care (POC) testing plays a crucial role in the context of the diagnosis and management of ACS. ACC/AHA UA/NSTEMI Guidelines have increased the need for rapid testing for cardiac biomarkers through a reduction in laboratory test turnaround time (TAT).[52] Decreased TAT is the central issue in POC testing. Recently, several devices for POC testing have been introduced.[53] Saliva-based nano-biochip using 21 biomarkers exhibited a significant diagnostic capability for AMI patients and has been proposed as a screening method for cardiac events in pre-hospital stages.[54] Our group recently developed a lab-on-chip system using small, highly sensitive microelectrodes that enable the detection of endothelial progenitor cells (EPC) at a low-level concentration (0.1% of EPC from 100 μL of blood) for the effective management of ACS patients.[55] However, these POC devices do have limitations and more trials are warranted. Moreover, it is important for emergency physicians and clinician assistants to understand the limitations of POC testing and make informed decisions based on the quality of the individual test platforms.

New Development in Biomarker Discovery

The traditional approach of biomarker discovery, which usually focuses on one or a few potential candidates at a time, has been ineffective and led to a low rate of biomarker discovery with clinical utility. The pathophysiologic changes in ACS are influenced by many factors, including genetic and environmental factors. The complete sequencing of human genome and recent advances in genomic, transcriptomic, proteomic, lipidomic, metabolomic, and bioinformatics technologies offer tremendous opportunities for novel biomarker discovery. These high-throughput technologies are emerging as powerful platforms for more global approaches to discover pattern-based unique molecular fingerprints or signatures of ACS.

At the genomic level, various global genomic technologies can be applied to study genetic variation, gene mutation, gene mapping, and epigenetic regulation. At the transcriptomic level, microarray technologies can be used to study the quantity of RNA and alternative splicing. With advances in proteomic technologies, the approach of biomarker identification now shifts from a large scale epidemiologic association to an association between disease phenotype and specific proteomic patterns. The proteomic approaches systematically study the identity, quantity, modification, localisation, and function of all the proteins in a high-throughput manner.

Combining new technologies paves the way for identification of novel biomarker protein signatures that can predict disease outcome more accurately. A recent report demonstrated that the 18 biomarkers extracted from a cytokine antibody array with bioinformatics analysis were able to predict patients who had mild cognitive impairment that progressed to Alzheimer's disease 2 to 6 years later.[56]

Using multimarker strategy could allow risk stratification and provide prognostic information in patients with ACS. A multimarker study combining baseline levels of cTnI, CRP and BNP among patients with NSTEMI identified a 6- to 13-fold gradient of mortality risk between those without elevation of any marker and those in whom all 3 markers were elevated.[57] Moreover, combined analysis of 10 biomarkers in 3209 participants attending the sixth examination cycle of the Framingham Heart Study showed that persons with high multimarker scores had a risk of death 4 times as great and a risk of major cardiovascular events almost 2 times as great as persons with low multimarker scores.[58]

Conclusion

Growing evidence has shown that the use of biomarkers reflects different pathologic entities, such as inflammation, oxidative stress, tissue necrosis, and platelet activation. However, no available biomarker offers ideal diagnostic properties for ACS, such as early detection, high sensitivity and specificity, easy availability, and cost effectiveness. Thus, the deployment of new strategies is essential to meet diagnostic, prognostic, and therapeutic needs. With the full use of newly emerging technologies, alone and in combination, novel biomarkers or novel biomarker protein signatures discovery is necessary. New development of

POC testing devices and use as a bed-side detection system are also essential for future management and patient care.

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