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Novel Biomarkers: Utility in Patients with Acute Chest Pain and Relationship to Coronary Artery Disease on Coronary CT Angiography

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Abstract Acute chest pain remains one of the most common patient presentations encountered in the emergency department. With the evolution of biomarkers and improvement in cardiac imaging there has been advancement in risk stratification of patients, but millions of dollars continue to be spent in the assessment of chest pain. Investigators have explored possible comparative alternatives to the traditional work up of chest pain. In this review, we will discuss the current state of biomarker use in the evaluation of acute chest pain. We will review established and emerging circulating biomarkers and their addition to cardiac CT for appropriate diagnosis of coronary artery disease.

Keywords Biomarkers · Cardiac CT · Coronary artery disease

Introduction

A major goal in the initial evaluation of a patient with chest pain is to be able to discern the acuity of symptoms and to triage appropriately. In the United States a large amount of expense and time is spent in the workup of acute chest pain in the emergency department (ED). It is estimated that as many as 8 million people per year visit the emergency department with chest pain with very few having myocardial ischemia from acute coronary syndrome as the etiology of their chest pain, but many undergoing traditional serial biomarker assessment which requires hospital admission or observation unit stay followed by diagnostic imaging, putting a tremendous amount of burden on the healthcare system [1]. This has led to multiple studies of established and emerging biomarkers in the acute coronary syndrome (ACS) cascade in the evaluation of patients with acute chest pain.

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History of the Diagnosis of Myocardial Infarction

Myocardial infarction (MI) was first diagnosed with the use of electrocardiogram (ECG) changes and history alone. The rational clinical exam has shown that ECG changes provide a tremendous amount of information regarding the increased likelihood of a MI, with new ST-segment elevation and Q wave as hallmark for acute coronary thrombosis and occlusion [2]. The diagnosis of ST elevation MI (STEMI) is clear by ECG alone, but diagnosing non-STEMI and unstable angina can be more difficult requiring additional data to risk stratify patients appropriately [3]. As such, the third universal definition of MI, published in 2012 states that the diagnostic criteria for MI requires a rise and/or fall of cardiac biomarkers (preferably troponins) with at least one value above the 99th percentile of the upper reference limit. In addition, patient should have symptoms of ischemia with new ECG changes and imaging evidence of a new loss of viable myocardium or new regional wall motion abnormality, or the identification of an intracoronary thrombus by angiography or autopsy [4].

Established Biomarkers in the Diagnosis of Acute Coronary Syndrome

The addition of creatine kinase (CK) allowed for more specificity of acute coronary syndrome (ACS), but sensitivity was low with only a small percentage of patients having a rise in CK activity and its lack of elevated levels did not precluded ACS. CK exists as isoenzymes, which are dimers of M (muscle type) and B (brain type) chains and exist in three combinations: MM, MB, and BB. Creatine kinase-MB fraction (CK-MB) was found to be more specific for myocardium with sensitivity 97 % and specificity 90 % [5]. However, CK-MB typically begins to rise four to six hours after the onset of infarction and is not elevated in all patients until about 12 hours. In more recent years, the use of CK and CK-MB has been surpassed by a more specific marker of myocardial injury and necrosis: troponin.

A more specific cardiac marker, troponin T (cTnT) is used to detect early myocardial ischemia and has become the mainstay of evaluation of acute chest pain patients. Troponin T is a protein of the cardiac contractile apparatus and is released into circulation with the death of myocardium. Beyond its diagnostic ability for MI, cTnT has prognostic value with greater elevations of cTnT associated with higher mortality and re-MI rates and a positive troponin at the time of presentation [6–9]. Troponin I (cTnI) was later found to have greater sensitivity and earlier detection of MI when compared to troponin T [10].

Although troponin T and troponin I are both expressed in cardiomyocytes and are released from the cytosolic pool into circulation after necrosis, they differ in biochemical and analytical characteristics. These differences include their proportion contained in the cytosolic pool, amino acid composition, molecular weight, time of increase after myocardial necrosis and, more importantly, their time of release after myocardial injury [11]. While minor differences are not relevant in patients with ACS, these differences may be amplified in patients with renal disease [12]. In renal impairment, cTnT is re-expressed in skeletal muscle and patients may have raised levels of cTnT in the absence of myocardial ischemia, making cTnI superior in this setting [13]. The discovery of troponin I having greater sensitivity for MI led to the evaluation of an accelerated chest pain protocol in the ED. In very low risk patients with Thrombolysis In Myocardial Infarction (TIMI) risk score of zero, an accelerated diagnostic ED protocol is feasible and safe, where patients after two negative TnI, 2 hours apart could be discharged [14]. Patients with TIMI score of zero are defined as having no ECG changes, without severe angina, less than three risk factors for coronary artery disease, no aspirin use within the last 7 days, and no history of significant coronary stenosis. This study is an example of how biomarkers alone can expedite the initial evaluation of very low risk patients with chest pain in the ED. However, in low-intermediate risk patients with symptoms suggestive of ACS, ED workup typically include either (1) serial troponin with provocative stress testing to exclude reversible ischemia, or more recently (2) a negative initial troponin with cardiac computed tomography angiography (cCTA) which is performed during the resting state to exclude obstructive CAD as the etiology of the acute chest pain [15••, 16••].

Coronary Computed Tomography Angiography in the Evaluation of Acute Chest Pain

The use of biomarkers in the evaluation of ACS has played a tremendous role in the management of patients that present with acute chest pain. However, with improvement in imaging capabilities, cardiac imaging has become integral in the assessment of patients with suspected ACS and serve as an invaluable adjunct to biomarkers. Initial cardiac imaging modalities were provocative in nature and required that the patient undergo stress imaging to evaluate for underlying obstructive coronary artery disease. CCTA to evaluate for coronary disease is a relatively new and promising imaging modality that is unique from prior forms of imaging in that it does not require stress provocation to determine burden of coronary artery disease.

Given the excellent predictive value of both biomarkers and CCTA, the ability to combine biomarkers with cardiac imaging would allow for improved risk stratification of patients and appropriate triage while decreasing the length of hospital stay at the same time. A meta-analysis of four randomized controlled trials had compared CCTA to standard care triage of acute chest pain in a total of 3266 low-to-intermediate risk patients who presented in the ED. It was noted that only 1.3 % overall MIs occurred mostly during the index hospitalization. In addition, length of hospital stay was significantly reduced with CCTA compared to standard care strategy. It was also found that CCTA significantly increased invasive coronary angiography (8.4 % versus 6.3 %) and revascularization (4.6 % versus 2.6 %). This meta-analysis included three major studies, CT-STAT [17••], ACRIN-PA [15••], and ROMICAT II [16••], trials which have been pivotal in the safe use of CCTA for early triage of patients in the ED [18••]. In each of these trials, patients with no ECG changes and a negative initial troponin were randomized to either CCTA or standard treatment with serial cardiac markers and ECGs.

The CT-STAT is a multicenter trial of low risk ED patients that prospectively included 749 patients who were either randomly allocated to CCTA (n =361) versus myocardial perfusion imaging (MPI) (n=338). Those in the CCTA arm had a 54 % reduction in time to diagnosis and 38 % reduction in costs. There was no difference in major adverse cardiac events (MACE) [17••]. The ACS rate was<3 %, thus a major

critique of this trial is that MPI was utilized in low risk patients that may not have warranted a nuclear stress test.

The ACRIN-PA multicenter trial was designed to evaluate the safety of CCTA in low risk patients in the ED [15••]. This trial included 1370 patients randomized in a 2:1 randomization assigned to CCTA versus standard of care. The trial concluded that utilization of CCTA early in the ED was safe with <1 % missed ACS (0 %, 95 % confidence interval 0-0.57 %). They also found that early CCTA led to a shorter mean hospital stay (18 versus 24.8 hours) and subsequently more frequent ED discharge when compared to standard of care (50 % versus 23 %) [15••].

The ROMICAT II trial is a multicenter comparative effectiveness trial randomizing early implementation of CCTA to standard ED evaluation in 1000 low-to-intermediate risk patients with suspected ACS (ACS rate 8 %) [16••]. The primary endpoint was length of stay. In the ROMICAT II trial, early CCTA utilization decreased the average length of stay in the hospital by 7.6 hours compared to standard ED evaluation. Additionally, there were no missed cardiac events within 72 hours, making CCTA a viable alternative for low-intermediate risk patients in the ED. However, increased diagnostic testing and higher radiation exposure was observed in the CCTA group. While there was a reduction in ED costs with an early CCTA strategy, there was no overall reduction in the cost of care during index hospitalization or 28-day follow-up.

In summary, all the above findings support the use of CCTA as an alternative to functional testing in low and low-intermediate risk patients with a single negative conventional troponin and non-ischemic ECG as an option to exclude obstructive epicardial coronary artery disease as the etiology of chest pain.

Emerging Diagnostic Biomarkers for Acute Coronary Syndrome

As biomarkers (Table 1, Fig. 1) have gained a greater role in the early assessment of chest pain, there has been a quest to discover novel biomarkers that can be used to quickly and accurately discern non-cardiac from cardiac chest pain.

Table 1 Biomarkers: role of biomarkers in the pathogenesis of atherosclerosis and ACS

Biomarkers	Mechanism of action
Biomarkers of necrosis:	
Creatine kinase – MB (CK-MB)	Enzyme specific to cardiomyocytes that converts creatine to phosphocreatine and adenosine diphosphate [5].
Troponin	Thin filament protein complex in myocardial and skeletal muscle important in muscle contraction [48].
Biomarkers of myocyte stress/stretch:	
B type natriuretic peptide / N-terminal pro-B type natriuretic peptide (BNP / NT-proBNP)	Protein important in decreasing systemic vascular resistance and increases natriuresis. NT-proBNP is biologically inactive and more stable than BNP and often used as a surrogate for BNP to measure ventricular stretch [49].
Mid-regional pro-atrial natriuretic peptide (MR-proANP)	Protein secreted into the circulation from both left atrial and left ventricular myocytes and is a measure of atrial stress [22].
Mid-regional pro-adrenomedullin (MR-proADM)	Adrenomedullin (ADM) is produced in many tissues and cell types including heart and the mRNA is expressed in endothelial cells. MR-proADM is the more stable form of ADM [22].
Copeptin	Pre-protein of vasopressin that is more stable than vasopressin and can serve as a surrogate. Vasopressin, antidiuretic hormone is important retention of water and increase in blood pressure [25•].
Biomarkers of inflammation:	
C-reactive protein (CRP)	Acute phase reactant that rises in response to inflammation [50].
Vascular cell adhesion molecule (VCAM)	Adhesion molecule expressed by endothelial cells [31].
Matrix metalloproteinase (MMP)	Zinc dependant endopeptidase that degrade extracellular matrix proteins [35•]. The MMPs family include gelatinases, MMP-2 (Gelatinase-A) and MMP-9 (Gelatinase-B). MMP-2 is encoded by the MMP2 gene while MMP-9 by the MMP9 gene. These genes encode an enzyme which degrades type IV collagen in basement membranes and extracellular matrix [33, 34].
Myeloperoxidase (MPO)	Peroxidase enzyme expressed by neutrophil granulocytes [38•].
Biomarker of fibrosis:	
Galectin-3	Beta-galactoside binding protein released from macrophages and endothelial cells that serves as an inflammatory marker and associated with fibrosis [41].

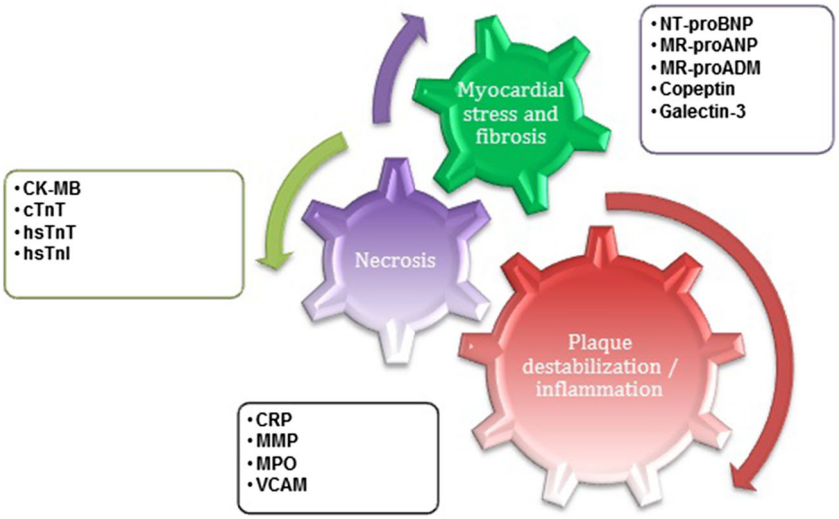

Fig. 1 Pathogenesis in acute coronary syndrome and the role of biomarkers

Biomarkers of Necrosis Most recently, a more sensitive biomarker of troponin is high sensitivity hs-troponin, which may be either high sensitivity troponin T (hsTnT) and high sensitivity troponin I (hsTnI), depending on the assay used. The benefits of hs-troponin are two-fold: (1) earlier detection than conventional troponin, and (2) the ability to detect below the limits of the conventional troponin assay; though at the expense of loss in specificity. HsTnT has 96 % negative predictive value for ACS and hsTnT above the 99th percentile has a nine-fold increase in predicting ACS [19]. HsTnT could be used as a way to rule out ACS in patients with undetectable cTnT, thus leading to improved risk stratification in low risk patients [20••]. This has been corroborated by the studies that showed hsTnT provided greater sensitivity as compared to conventional troponins because of the ability to detect much smaller quantities of myocardial injury in ACS among low to intermediate risk patients. However, hsTnT showed moderate sensitivity particularly for the diagnosis of unstable angina (UAP) [21•].

Biomarkers of Myocardial Stress/stretch Given the still imperfect diagnostic accuracy of using a single marker strategy of troponin, a dual marker strategy for ACS and UAP diagnosis by the addition of natriuretic peptides to both conventional and highly sensitive methods for troponin have been suggested [21•]. The natriuretic peptides, including N-terminal pro-B type natriuretic peptide (NT-proBNP) and the newer mid-regional pro-atrial natriuretic peptide (MR-proANP) and mid-regional pro-adrenomedullin (MR-proADM), are markers of myocyte stress secreted into the circulation via atrial and ventricular myocytes and they have been shown to correlate well with cardiac structure and function [22]. Potentially using a dual marker approach may have incremental value and could be superior to a single troponin strategy alone for ACS and UAP diagnosis [21•]. The combination of NT-proBNP and hsTnI added diagnostic information to cTnT [23•]. Patients with increased cTnT and proBNP have increased adverse events including death, new ACS, revascularization, and heart failure at 6 months adding useful information to the TIMI risk score [24].

Another emerging biomarker studied is copeptin in ED patients with symptoms suggestive of ACS [25•, 26•, 27]. Copeptin is a portion of the precursor protein to vasopressin (AVP). Given the short half-life of AVP, copeptin has been suggested as a surrogate for AVP. Physiologically AVP is thought to reflect vascular tone changes with an acute MI and is found to rise in the setting of an MI. The CHOPIN trial [25•] investigated the use of copeptin as a biomarker to rule out MI in conjunction with troponin T. The use of both biomarkers had a negative predictive value >99 %, particularly in patients who presented within 3 hours of symptoms [25•]. The addition of copeptin to cTnT and hsTnT may improve the diagnostic accuracy for ACS compared to the use of cTnT alone [28]. As with the development of hsTnT there has now been assays designed for ultrasensitive (us)-copeptin to detect copeptin levels at even lower values than the traditional copeptin assay. The combination of us-copeptin and hsTnT improved detection of AMI in the ED compared to hsTnT alone [26•]. However, there remains controversy over the use of copeptin as a biomarker for ACS detection [27].

Biomarkers of Inflammation C reactive protein (CRP) is a well-established marker of inflammation. Increased levels of high sensitivity hsCRP correlates with atherosclerotic plaque seen on CCTA [29] and found to correspond to plaques with increased necrotic core ratio among patients with ACS [30]. Moreover, the MIRACL study evaluated several inflammatory biomarkers and found CRP and interleukin-6 (IL-6) to be related to death but not recurrent ACS, while vascular cell adhesion molecule (VCAM) is related to both mortality and recurrent ACS [31]. VCAM is expressed on vascular endothelial

cells, an early feature in the pathogenesis of atherosclerosis and inflammation.

A more novel marker of inflammation, which may have implication in the ACS cascade, is matrix metalloproteinase (MMP). The MMPs family include gelatinases, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) which are encoded by the MMP2 and MMP9 genes respectively [32, 33]. These genes encode an enzyme which is important in the degradation of the extracellular matrix [33, 34]. Elevated MMP-9 was found to be an early indicator of ACS compared to hsTnT [35•]. Increased MMP-9 predicts future coronary revascularization in patients with acute MI [36].

Another inflammatory marker in the pathogenesis of plaque formation is myeloperoxidase (MPO), an enzyme expressed by neutrophils and monocytes [37]. MPO was found to add negative predictive value (NPV) to a negative cTnI in patients presenting with acute chest pain [37]. Elevated levels of MPO was found to have the highest discriminatory power when evaluated amongst a panel of biomarkers including high sensitivity c reactive protein (hsCRP), oxidized low density lipoprotein (oxLDL), and MPO in patients with ACS [38•]. In the EPIC prospective study, MPO was been found to be able to predict future risk of CAD in healthy individuals [39].

Biomarkers of Fibrosis Galectin-3 is a ubiquitous marker associated with myocardial fibrosis, and released from macrophages and endothelial cells [40, 41]. The burden of coronary artery disease may be correlated with higher levels of galectin-3 especially in unstable angina [41].

Relationship of Biomarkers to CT Angiography

Coronary Plaque Morphology and Biomarkers Plaque formation begins with increased intimal permeability allowing for the entry of low density lipoproteins (LDL) into the endothelium. LDL is then oxidized with later recruitment of leukocytes to the vessel wall. This is followed by ingestion of oxidized LDL to form foam cells. Smooth muscles migrate to form fatty streaks and a fibrous cap that is made from the extracellular matrix. Hemodynamic stresses and degradation of extracellular matrix increases the susceptibility of the fibrous cap to rupture, allowing superimposed thrombus formation [42].

Plaque composition is thought to play an important role in predicting which ones are most vulnerable to rupture and has important value not only in the discovery of biomarkers but also in CCTA evaluation. Culprit plaques for acute coronary syndrome found on autopsy have been those with a thin fibrous cap, large necrotic core, high lipid content, and high macrophage count [43, 44]. Atherosclerotic plaques can be classified into calcified, non-calcified or mixed. Non-calcified plaque is any structure having a CT density of<130 Hounsfield Unit (HU) while calcified plaque is any structure with a density of >130 HU, that could be assigned to the coronary artery wall, and that could be identified in at least two independent planes [45•]. Mixed plaque is defined as the presence of both non-calcified and calcified plaque [45•]. Investigators have found that hs-CRP and MMP-2 were positively correlated with the extent of calcified plaque, while levels of hs-CRP were positively correlated with the extent of both, non-calcified and calcified plaque [45•]. Another study found that patients with an elevated hsTnT were more likely to have an abnormal nuclear imaging. This correlated to mixed plaques seen on CCTA, which are thought to be from plaques most vulnerable to rupture during ACS [46•].

Cardiac CT Morphology and Biomarkers Biomarkers have been found to correlate with cardiac imaging. Patients found to have good cardiac health assessed by cardiac CT and nuclear imaging had lower levels of hsTnT and hsCRP [29]. HsTnT correlated with myocardial perfusion abnormalities and LV hypertrophy/dysfunction, while hsCRP correlated with atherosclerotic plaque seen on CCTA [29]. Natriuretic peptides including both NT-proBNP and MR-proANP are associated with CT-metrics of LA enlargement, whereas MR-proADM is negatively correlated with LV volumes [22]. These studies provide some mechanistic insight and relationship between circulating biomarkers and cardiac morphology.

Conclusion

The advent of novel biomarkers has the potential for improved diagnostic capabilities over traditional biomarker assays for the evaluation of acute chest pain. CCTA has been shown to reduce length of stay in the hospital and is an alternative to standard ED evaluation of acute chest pain. It remains unclear how implementing newer biomarkers, such as hsTnT, will affect the evaluation of patients with acute chest pain such as in the United States, where the Food and Drug Administration has still not approved a hsTnT test [47]. Would it lead to subsequently more testing and increase costs due to its lower specificity? Or would it lead to less downstream testing and reduce costs because of the ability to expedite accelerated protocols to exclude ACS? These unanswered questions make this an exciting, dynamic time in cardiac imaging and biomarkers. Chest pain is so commonly encountered in the practice of medicine and we are on the cusp of changing the face of how ACS is diagnosed and managed.

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Compliance with Ethics Guidelines

Conflict of Interest Adefolakemi Babatunde and Asim Rizvi declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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