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Novel Risk Stratification Assays for Acute Coronary Syndrome

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Abstract

Purpose of Review Since identification of aspartate aminotransferase as the first cardiac biomarker in the 1950s, there have been a number of new markers used for myocardial damage detection over the decades. There have also been several generations of troponin assays, each with progressively increasing sensitivity for troponin detection. Accordingly, the "standard of care" for myocardial damage detection continues to change. The purpose of this paper is to review the clinical utility, biological mechanisms, and predictive value of these various biomarkers in contemporary clinical studies.
Recent Findings As of this writing, a fifth "next" generation troponin assay has now been cleared by the US Food and Drug Administration for clinical use in the USA for subjects presenting with suspected acute coronary syndromes. Use of these high-sensitivity assays has allowed for earlier detection of myocardial damage as well as greater negative predictive value for infarction after only one or two serial measurements. Recent algorithms utilizing these assays have allowed for more rapid rule-out of myocardial infarction in emergency department settings.
Summary In this review, we discuss novel assays available for the risk assessment of subjects presenting with chest pain, including both the "next generation" cardiac troponin assays as well as other novel biomarkers. We review the biological mechanisms for these markers, and explore the positive and negative predictive value of the assays in clinical studies, where reported. We also discuss the potential use of these new markers within the context of future clinical care in the modern era of higher sensitivity troponin testing. Finally, we discuss advances in new platforms (e.g., mass spectrometry) that historically have not been considered for rapid in vitro diagnostic capabilities, but that are taking a larger role in clinical diagnostics, and whose prognostic value and power promise to usher in new markers with potential for future clinical utility in acute coronary syndrome.

Keywords Biomarkers · Troponin · Acute coronary syndrome · Myocardial infarction · Biological assay

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Introduction

Cardiovascular disease remains the leading cause of death in the USA and worldwide [1]. Accordingly, early and accurate diagnosis of acute coronary syndrome (ACS) is a critical step for improving patient health outcomes. Approximately 6 million Americans present to emergency departments (EDs) with chest pain every year [2]. Symptoms, vital signs, electrocardiograms (ECGs), and various lab assays may be used to help triage patients toward discharge, continued monitoring, medical therapy, and invasive interventions such as cardiac catheterization.

For the past few decades, assays used to triage subjects with ACS, like cardiac troponins, focused on the detection and quantification of myocardial specific proteins released into the systemic circulation after myocardial cell necrosis. Successive improvements to these assays enable ever-

increasing sensitivity to detect myocardial injury and necrosis, shortening the time between onset of ischemic insult and time to accurately detect and diagnose myocardial infarction (MI). In addition to classic markers of myonecrosis, new technologies have enabled discovery of novel cardiovascular biomarkers that are increasingly showing added clinical prognostic value. However, many questions remain regarding the diagnostic accuracy and additive outcome discrimination for these assays. In this review, we discuss the novel assays available for ACS detection including high-sensitivity cardiac troponin (hs-cTn), the predictive value of these assays, and how they compare to the current standard of care. We also speculate on the potential for mass spectrometry-based platforms playing an ever-larger role in cardiovascular disease diagnostics, including within the rapid time frame demands of subjects presenting with ACS.

Assays for Detection of Acute Coronary Syndrome

Cardiac Troponin

Cardiac troponins (cTn) are proteins mainly distributed in the sarcoplasmic reticulum of a cardiac myocyte with small amounts also found in the cytoplasm. The cTn complex is comprised of three subunits—an inhibitory component (TnI), a tropomyosin binding component (TnT), and a calcium binding component (TnC) [3]. TnI and TnT are specific for cardiac myocytes and are, therefore, used as markers for cardiac injury and myonecrosis. They are released within 2 h of symptom onset, peak at 12 h, and remain elevated for 5–14 days [4]. Despite their specificity to cardiac muscle, cTn may still be detected in a number of pathological states other than ACS including hypertensive emergency, stroke, sepsis, chronic kidney disease, gastrointestinal bleeding, and rhabdomyolysis. In these situations, it is possible that the small amount of troponin in the cytoplasm is released during high myocyte turnover and due to increased cell wall permeability [5, 6]. For this reason, it is important for cTn to be interpreted in the context of high clinical suspicion for ACS.

Currently, use of cTn assays for detection of cardiac injury has become the standard of care, and over the years, the sensitivity of these assays has progressively increased. First-generation cTn assays had diagnostic cutoffs of 500 ng/L for detection of MI [7]. These cutoffs have since decreased to 10 ng/L with more contemporary assays and have limits of quantification down to as low as 1 ng/L in hs-cTn assays. High-sensitivity assays for both cardiac-specific troponin I or T are currently available and under investigation.

On balance, studies evaluating hs-cTn have found increased sensitivity and negative predictive value (NPV) for MI detection at the expense of specificity and positive predictive value (PPV). A meta-analysis by Lipinski et al. showed

that troponin lab draws 2.5 h after admission showed higher sensitivity for MI but lower specificity than contemporary cTn assays [8]. Aldous and colleagues compared hs-cTnT (Roche Elecsys), third-generation TnI (Abbott Diagnostics, Abbott Park, IL, USA), and contemporary TnT (Roche Diagnostics, Rotkreuz, Switzerland) in chest pain patients at initial presentation to the ED and at follow-up [9]. They evaluated area under the receiver operating characteristics curve for MI and found it to be superior at baseline when using the hs-cTnT and third-generation TnI compared to the contemporary TnT assay. An increase in troponin ≥20% after median 9 h increased specificity (81 to 94%) of the hs-cTnT assay at the cost of lower sensitivity (91 to 72%). Others studying the introduction of hs-cTnT (Roche Elecsys 2010) to an ED previously using contemporary TnT assays showed similar results with an increase in MI diagnoses and decrease in unstable angina diagnoses when using the hs-cTnT assay [10].

Having established how sensitive these hs-cTn assays are, adding a second diagnostic tool can increase NPV even further to effectively rule out low-risk patients. Bandstein and colleagues collected ECG and hs-cTnT (Roche Elecsys 2010) data from 14,636 patients with chest pain who presented to the ED in Sweden [11]. Of those patients, 8907 had no detectable troponin elevation and no ischemic ST changes. At 30 days, 15 patients had an MI and two died, yielding a NPV of 99.8 and 100%, respectively, although this may have been an unusually high undetectable rate. In a recent meta-analysis of 9241 presenting to the ED with chest pain, pooled sensitivity for MI detection was 98.7% (95% confidence interval 96.6–99.5%) and for major adverse cardiovascular events (MACE) at 30 days was 98.0% (94.7–99.3%) [12•].

With the development of hs-cTn assays, studies have increasingly tried to characterize the appropriate timing of troponin measurement. One study evaluated a 1-h algorithm using hs-cTn (Roche Elecsys 2010) at presentation and at 1 h, along with symptoms, physical examination, and ECG in 1320 patients presenting with chest pain to multiple centers [13]. MI rule-out was defined as a troponin <12 ng/L and a change of <3 ng/L at 1 h. MI rule-in was defined as >52 ng/L measurement at baseline or an absolute increase of ≥5 ng/L. Of the 1320 patients, 786 patients were ruled out based solely on the troponin which equates to a 99.9% NPV. None of those patients died within 30 days. However, the number of early-presenting patients in that study was limited.

In a larger, more recent study of 2222 patients presenting with chest pain, those with serial hs-TnT using a 1-h algorithm had a lower sensitivity of 97.1% for acute MI which may be insufficient [14]. In that study, the investigators obtained the second troponin 3 h after the first one in patients presenting very early (symptoms less than 1 h), but this did not change the number of false negative patients who were ruled out for MI. Some have argued for a miss rate of <2% for acute MI in the ED, while the authors have argued for a lower rate of ≤1%.

In that study, the miss rate was higher than both thresholds when using the 1-h algorithm. Therefore, experts have called for further investigation of such algorithms in early-presenting patients [15].

The APACE study included patients presenting to European EDs with chest pain and Thrombolysis in Myocardial Infarction (TIMI) risk scores of zero to one using hs-TnI and ECG [16]. NPV for MI in patients with TIMI score 0 was 100%, and those with TIMI score 1 was 99.7%. Similarly, the TRAPID-AMI study evaluated 1282 patients presenting with chest pain across multiple centers in Europe, Australia, and the USA [17•]. Hs-cTnT (Roche Diagnostics) and hs-cTnI (Siemens Healthcare) were measured at baseline and 1, 2, and 4 to 14 h after presentation. A 0-h/1-h algorithm was used to rule out or rule in patients for MI. Among 1282 patients enrolled, 813 (63.4%) were classified as "rule-out" with a NPV of 99.1%, and 184 (14.4%) were classified as "rule-in" with a PPV of 77.2%.

Two-hour algorithms have also been evaluated and validated for MI categorization. In 385 patients presenting with chest pain, the NPV of MI using a 2-h algorithm utilizing hsTnT was 98.3% with a PPV of 53.8% [18]. Adding ECG results reduced the false negative rate to 1.2%. Other researchers have found that hs-cTn samples collected 4 to 6 h after symptom onset yields 100% sensitivity for MI with more accurate prediction of death, heart failure, and nonfatal MI than contemporary cTn assays [19, 20].

Based on the previous algorithms, the European Society of Cardiology (ESC) had included in their guidelines 0-h/1-h algorithms utilizing hs-cTn assays for patients presenting with chest pain [21••]. However, the increased sensitivity of these assays comes at the cost of specificity, which has raised questions regarding the potential for increased false positives, and resultant unnecessary additional diagnostic testing and procedures. Haider and colleagues retrospectively analyzed hs-TnT (Roche Diagnostics, Switzerland) results of 1573 patients admitted to a university hospital ED with chest pain [22]. They found the sensitivity for detection of ACS to be >92%, but the specificity to be approximately 35%. They also tested the ESC algorithm for detection of MI and observed a sensitivity of 83%, while 43% of non-MIs were incorrectly classified as MIs. A more recent study assessing the validity of the ESC algorithm in patients presenting with chest pain in Australia, New Zealand, and Canada showed a PPV of 63% for hs-TnT and 68% for hs-TnI [23]. Based on these results, the authors have raised concerns that hs-Tn assays lack adequate specificity to distinguish between ACS and myocardial damage of other origin even when the ESC algorithm is applied.

Others have argued that hs-cTn elevation has prognostic value and mortality discrimination despite its low specificity. Potter and colleagues studied the distribution of hs-cTn elevation across hospital and community outpatient settings [24]. They found that patients on medical and surgical wards without a primary cardiac diagnosis and hs-cTn >99th percentile had a highly significant odds ratio of death. Irrespective of these data, clinicians must always be cognizant of the negative and positive predictive values of these assays when interpreting results. With the expected dissemination of hs-cTn assays in the USA, it is important to utilize the history, physical examination, ECG, and possibly risk scores to help correctly categorize patients with chest pain.

Although hs-cTn assays have been used in Europe for many years, they had not yet been available for clinical use in the USA until 2017. As of the time of this writing, the US Food and Drug Administration (FDA) has newly issued clearance for the Troponin T Gen 5 STAT assay (Roche Diagnostics) [25••]. This clearance includes the Troponin Gen 5 STAT CalSet, PreciControl Troponin, and Troponin T Gen CalCheck 5. This is a fifth-generation immunoassay for the quantification of cardiac troponin T. It is run using lithium heparin plasma and is intended for the diagnosis of MI. The assay can be run on the Roche cobas e411 e601, and e602 autoanalyzers, and is reported to take approximately 9 min to run, so thus can support the rapid time demands of an ED-based testing platform. The FDA has advised labeling it a "next generation" as opposed to "high sensitivity" troponin assay, but it will be the same test referred to as hs-cTn in published studies and in Europe. This is the first of many higher sensitivity myocardial-specific troponin assays that are seeking FDA clearance in the USA, and marks the beginning of a new era in early MI diagnosis and prognostication.

Creatinine Kinase and CK-MB

Creatinine kinase (CK) is comprised of three isoenzymes—MM, BB, and MB. CK-MB is primarily present in cardiomyocytes and is rapidly released after cardiac injury [26]. During an MI, CK-MB doubles within 6 h and reaches a peak within 12–24 h [27, 28]. CK-MB measurements have an approximately 90% sensitivity for detection of MI 6 h after symptom onset, but the sensitivity goes down to approximately 35–50% when used sooner such as on presentation or 3 h after symptom onset [28–30]. Since the newer generation troponin assays have higher sensitivity, they have now largely replaced the use of CK-MB assays for MI detection. Therefore, if a troponin assay is available, then the CK-MB simply adds cost without additional diagnostic value. However, if a cTn is not available, the CK-MB still remains an excellent alternative marker. It can also play a role in defining infarct size, infarct expansion based on serial measurement, and risk of reinfarction [31].

Like troponin, CK takes several hours to be released from necrotic myocardial cells, and may be elevated in several conditions other than MI. While the MB isoform is selectivity enriched in cardiomyocytes, CK is also enriched in the intestine, prostate, uterus, and diaphragm, so extensive injury to

these organs may also increase the CK-MB, thereby reducing the specificity for cardiac damage. Clinical context and examination of the proportion of CK-MB vs total CK can help with interpretation. In the setting of suspected ACS, CK-MB is thus sometimes measured as a percentage of total CK. However, several professional societies including the ESC, American College of Cardiology, and World Health Organization still recommend use of absolute CK-MB levels based on one or two consecutive measurements [31, 32].

Myoglobin

Myoglobin is a small heme protein found in the cytoplasm of cardiac and skeletal myocytes [33]. It is mainly used to transport oxygen within muscle cells, and is rapidly released upon muscle injury. Myoglobin is one of the most rapidly detected biomarkers after MI, since it appears in the circulation after 30 min to 2 h. However, it is relatively nonspecific since cardiac and skeletal myocytes share 100% homology.

Guidelines recommend myoglobin measurements only in patients presenting within 6 h of chest pain onset [34]. For patients presenting with chest pain, the addition of myoglobin to CK-MB or contemporary cTn early after symptoms onset was shown to improve sensitivity for MI detection in multiple studies [27, 28, 30, 33]. However, with the development of highly sensitive cTn assays, the value of myoglobin as a cardiac biomarker is unclear, and needs to be examined.

Trimethylamine N-Oxide

Trimethylamine N-oxide (TMAO) is a plasma metabolite of trimethylamine and is formed through a metaorganismal pathway involving gut microbiota [35, 36]. It was originally discovered based on untargeted metabolomic studies whose goal was to identify novel pathways linked to cardiovascular disease risks, and then was subsequently shown to be mechanistically linked to cardiovascular disease pathogenesis and heightened thrombosis potential [35–43]. In recent years, numerous studies have shown an association between this novel metabolite and cardiovascular risks [35, 37–43]. Human and animal studies showed that TMAO interacts with platelets and alters calcium signaling, thereby enhancing platelet reactivity and thrombosis [39]. TMAO was also shown to promote vascular inflammation and endothelial cell activation in animal models [44].

Based on the aforementioned mechanisms, TMAO elevation would be expected to be associated with increased CVD event and thrombosis risks. Indeed, emerging data shows that within patients presenting to the ED with a complaint of chest pain, initial baseline TMAO levels were highly predictive of both near-term MACE including MI, stroke, need for revascularization, and death, and long-term mortality risks. For example, among patients presenting with chest pain to the

Cleveland Clinic, as well as in a replication multisite clinical study in Switzerland, elevated plasma TMAO was independently associated with incident near and long-term MACE risk [45]. In the Cleveland Clinic cohort ($n = 530$), plasma TMAO level at presentation predicted risk of MACE over the ensuing 30 days (Q4 adjusted OR 5.83, 95% CI, 1.79–19.03; $p < 0.01$) even among patients with no detectable troponin by hs-TnT assay (Roche fourth-generation assay) at baseline presentation, as well as among those who remained persistently negative for TnT [45].

Having already discussed the incredibly high NPV for hs-TnT assays which often exceeds 99%, it is particularly striking for an alternative marker to have additive discrimination beyond the fourth-generation troponin assays. Current TMAO testing is used in the clinical setting of stable patients, since the platform for its measurement (mass spectrometry) is not currently available for more urgent settings. However, as great advances in the technology continue to accrue, and increasingly the mass spectrometry platform is entering the clinical laboratory for in vitro diagnostics, its potential for use in risk stratification of subjects presenting with chest pain of suspected ACS origin will be interesting to follow.

Brain Natriuretic Peptide

Brain natriuretic peptide (BNP) is a polypeptide cardiac neuro-hormone that is secreted from membrane granules in the left ventricle as a response to volume and pressure overload [46, 47]. BNP levels often increase in various cardiac and noncardiac conditions including MI, heart failure, pulmonary hypertension, renal failure, and cirrhosis, and may be used to gauge symptom severity and prognosis [48]. Amino terminal pro-brain natriuretic peptide (NT-proBNP) has been cleared by the FDA for assessing the prognosis of patients with congestive heart failure and ACS [48]. The BNP assay is also cleared by the FDA for risk stratification in ACS [49].

Despite the FDA clearance of BNP and NT-proBNP assays, its prognostic utility in ACS remains questionable. BNP and NT-ProBNP have been diagnostic of death in most ACS studies, but the prediction of recurrent MI is less clear [4]. Also, there is significant heterogeneity in the cutoffs used for these assays and in the necessary actions that should be followed based on the result. The TACTICS-TIMI trial showed that women with elevated BNP seemed to benefit from early invasive strategies even when cTn was normal [50]. On the other hand, another smaller study of patients presenting with ST elevation MI showed there was similar prognostic value for the prediction of multivessel coronary artery disease with hs-cTnT, CK, high-sensitivity C-reactive protein (hs-CRP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine transaminase (ALT) peak concentrations (AUC = 0.77, 0.77, 0.68, 0.79, 0.78, and 0.73, respectively) [51]. In fact, the prognostic utility of

NT-proBNP was lower than the other markers (AUC = 0.64), and no better than hs-cTnT ($p = 0.349$). For these reasons, NT-proBNP and BNP assays are often used for congestive heart failure assessment but still not routinely used for ACS per se.

Ischemia-Modified Albumin

The N-terminal portion of serum albumin is a binding site for transition metal ions including cobalt, nickel, and copper [52]. Ischemia-modified albumin (IMA) is a form of serum albumin where the N-terminal amino acids are unable to bind these transition metals possibly due to modifications to the protein that occur during ischemia [53]. IMA can be measured using an albumin cobalt binding assay. In MI, IMA levels rise within 6 h and remain elevated for 12 h. A meta-analysis of patients presenting to EDs with chest pain showed that a combination of IMA, ECG, and contemporary troponin assays had a NPV of 97% for MI [54]. However, the NPV for IMA on its own is significantly lower. In 2010, the US FDA cleared IMA as a marker to be used in combination with cTn and ECG for diagnosis of ACS. Its use, however, has not yet been shown to provide added value to contemporary hs-TnT assays.

High-Sensitivity CRP

C-reactive protein is an acute-phase reactant produced in the liver and is related to various functions including complement activation and phagocyte stimulation [55]. C-reactive protein (CRP) has been found to correlate with plaque vulnerability, angina, MI, coronary vasospasm, and left ventricular dysfunction [56–60]. However, the routine use of CRP levels in acute MI detection has several limitations. Human CRP levels vary greatly depending on gender, ethnicity, age, and genetics [61]. Moreover, it is often used as a marker for noncardiac pathologies such as systemic lupus erythematosus and dementia [62, 63]. For these reasons, it is not routinely used for acute MI detection.

Lipoprotein (a)

Apolipoprotein A is linked to low-density lipoprotein (LDL) by a disulfide bond to create lipoprotein (a) (LP(a)) [64]. LP(a) enhances thrombosis risk and increases the atherogenicity of LDL [65, 66]. Several observational studies have shown an association between LP(a) and both CVD and thrombosis risk [67]. However, no studies have shown it to have added prognostic or diagnostic utility in ACS beyond the high-sensitivity troponin assays.

Myeloperoxidase

Myeloperoxidase (MPO) is an enzyme released early in the inflammatory process and has been shown to be related to CVD due to its involvement in nitric oxide catabolic consumption, and oxidative modification of lipoproteins in the artery wall including both high-density lipoprotein and LDL [65, 66]. Multiple cross-sectional and prospective studies have shown MPO to be associated with improved CVD risk stratification, though most of these studies included contemporary cTn assays as opposed to hs-cTn [68–73]. MPO levels recovered from patients following ST segment elevation MI have been shown to predict death, reinfarction, and the need for coronary revascularization [69]. Serial testing with MPO in patients who presented to the ED with chest pain was shown to predict outcomes up to 16 h after presentation, and predicted events missed by cTn testing [68, 72].

In another recent study, multiple biomarkers of plaque destabilization and disruption were examined, including MPO, high-sensitivity interleukin-6, myeloid-related protein 8/14 (MRP8/14) and pregnancy-associated plasma protein-A (PAPP-A) among consecutive patients presenting with stable nonobstructive coronary artery disease (CAD), stable obstructive CAD, and non-ST segment MI [70]. Compared to other biomarkers, MPO had the highest specificity (83%) for NSTEMI diagnosis ($p < 0.05$). These studies support a potential role for MPO in augmenting TnT or TnI in the rapid triage of patients presenting to the ED with chest pain and suspected ACS. Further studies in the high-sensitivity cTnT era with MPO are needed. If MPO is to be drawn, the preferred anticoagulant and collection tubes are EDTA or EDTA plasma preparation tubes, as opposed to heparin plasma tubes [74].

Uric Acid

Heightened levels of uric acid have long been associated with both the presence and the severity of CAD [75]. However, its role in acute diagnostic settings, such as ACS, remains unclear. A study of patients presenting with chest pain found that subjects with ST elevation MI had higher uric acid levels than those with non-ST elevation MI or unstable angina ($p < 0.05$) [76]. Uric acid was then also higher in unstable angina patients than in patients with chest pain and normal coronary arteries ($p < 0.05$). This demonstrates progressive discrimination in ACS severity based on uric acid level. Another similar cross-sectional study showed serum levels of uric acid to be higher in ACS patients compared to apparently healthy controls ($p < 0.01$) [77]. However, these studies did not compare the additive value of uric acid to traditional cardiac biomarkers.

In a recent single site study of 951 sequential patients presenting with chest pain, uric acid was examined for its potential clinical utility to identify subjects with ACS [78]. While elevated uric acid level was useful for identifying ED patients with chest pain who are at high risk for 1-year mortality, it lacked diagnostic utility for ACS. Thus far, uric acid has not been used for ACS determination in routine clinical practice.

Table 1   Point-of-care panel assays for acute coronary syndrome

	cTn	CK-MB	Myo	Volume (drops)	Sample type			Time (min)	Approval
					WB	P	S		
Decision Point (EU)/Cardiac STATus (USA) (Nexus Dx, Inc., USA)	●	●	●	6	●	●	●	15	FDA/CE
RAMP (Response Biomedical, Canada)	●	●	●	6	●			15	FDA/CE
Nano-Check Cardiac Markers (Nano-Ditech Corp., USA)	●	●	●	6	●	●	●	10–15	FDA
Stratus CS Acute Care (Siemens AG, Germany)	●	●	●	6	●			14	FDA
MI CK-MB/Myo/TnI (LifeSign LCC, USA)	●	●	●	3	●	●	●	15	CE
Instant View (Alfa Scientific. Inc., USA)	●	●	●	4	●		●	10	CE
Triage Cardiac Panel (Alere, UK)	●	●	●	6	●	●		15	CE
Cobas h 232 System (Roche Diagnostics Ltd., UK)	●	●	●	4	●			8–12	CE
Cardiac Marker Tests (Anibiotech, Finland)	●		●	3	●		●	15	CE
Cardiac STATus (Spectral Diagnostics Inc., Canada)	●	●	●	6	●	●	●	15	
AQT90 FLEX Analyser (Radiometer Ltd., England)	●	●	●	6	●	●		10–20	
Vidas Emergency Panel (BioMerieux Ltd., UK)	●	●	●	6	●	●	●	30	
Pathfast (Mitsubishi, Tokyo)	●	●	●	3	●	●	●	16	

Adapted from the National Institute for Health Research Diagnostic Evidence Co-operative [111]

cTn cardiac troponin, *CK-MB* creatinine kinase MB, *Myo* myoglobin, *WB* whole blood, *P* plasma, *S* serum

Other Novel Biomarkers

Asymmetric dimethylarginine (ADMA) is a competitive inhibitor of nitric oxide synthase and has been found to be elevated in multiple cardiac conditions including hypertension, heart failure, and CAD [77–82]. In a study of 1011 sequential subjects presenting for diagnostic coronary angiography and monitored for incident 3-year risk of MI, stroke, or death, elevated levels of ADMA were a significant independent predictor (adjusted HR 2.2, 95% CI 1.2 to 4.0, $p = 0.015$) of major adverse cardiac events [83]. It has also been reported that ADMA levels are associated with fatal MI, but its utility in ACS and acute MI detection has yet to be demonstrated [84].

Soluble ST2 is an interleukin family biomarker encoded by the IL1RL1 gene. It is elevated during times of cardiac fibrosis and adverse remodeling including during MI and worsening heart failure [85, 86]. Early studies have shown patients presenting with ST segment elevation MI and ST2 levels higher than 35 ng/mL to have more than three times higher cardiovascular death and new heart failure incidence at 30 days [87]. ST2 can be measured using a commercially available immunoassay and is cleared by the US FDA (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID= 36927). In a recent study, the prognostic value of sST2 compared with hs-cTnI was examined among sequential patients presenting to the ED with chest pain [88]. Interestingly, soluble ST2 levels at time of presentation showed higher independent predictive power than hs-cTnI for cardiovascular events. Further examination of this new marker in ACS subjects seems warranted.

C-terminal provasopressin (copeptin) is a glycosylated peptide synthesized in the hypothalamus and contributes to the folding of vasopressin [89, 90]. It is secreted during hemodynamic and stress-related stimuli such as acute MI [91, 92]. Since it is released very early on during MI, it has been suggested to be useful in ruling out MI along with troponin and has a NPV >95% in clinical studies [91–94]. In a recent study of 637 sequential subjects presenting to the ED with chest pain, addition of copeptin to hs-cTn was found to not provide significant improvement in the diagnostic accuracy of ACS [95].

Placental growth factor (PIGF) is another marker in the vascular endothelial growth factor family. It was previously shown to be upregulated in atherosclerosis, so researchers last

decade had hoped that it may have utility in ACS [96]. In one study, Heeschen et al. collected PlGF levels from 547 patients with confirmed ACS [97]. At 30 days follow-up, patients with increased PlGF levels had a significantly higher risk of death and MI independent of TnT. However, to our knowledge, recent studies have not shown an independent and additive value beyond the higher sensitivity cTn assays. As a result, assays to date for the measurement of PlGF have mostly been used for research purposes.

Pregnancy-associated plasma protein A (PAPP-A) is a zinc-binding metalloproteinase which has been shown to be associated with coronary plaque [98]. Bayes-Genis and colleagues examined the expression of PAPP-A from eight patients who died suddenly of cardiac causes [99]. Circulating PAPP-A levels were significantly higher in the ACS patients than in controls ($p < 0.001$). PAPP-A elevation identified patients with ACS, but with only a sensitivity of 90% and specificity of 81%. Later, Heeschen and colleagues found that PAPP-A identified patients at high risk of death and MI (odds ratio 2.7 with 95% confidence interval 1.3–5.9) even when TnT was negative [100]. However, recent studies have shown that concomitant heparin administration increases PAPP-A levels and delays its clearance from the circulation [101]. Further studies are needed to determine the predictive value of this marker in heparin-naive patients.

Heart-type fatty acid binding protein (H-FABP) is a protein encoded by the FABP3 gene and is 20 times more specific to cardiac muscle than myoglobin [102, 103]. It can be detected within 1 to 3 h of the onset of MI [102, 104, 105]. In one study involving 1128 subjects presenting with chest pain, measurement of H-FABP along with cTn was shown to accurately diagnose MI with a negative predictive value of 98% [106]. It was also shown to have added survival discrimination beyond cTn. In patients with negative TnI but positive H-FABP there was a 17% increased risk of all-cause death at 1 year compared to patients who were TnI positive [107]. However, the additive value when compared to the newer generation cTn assays remains unclear.

Finally, hydrogen sulfide (H_2S) is a gasotransmitter, formed both in the gut and endogenously, that has been reported to have effects on multiple organ symptoms including the blood vessels and heart [108]. Early studies have preliminarily shown potential molecular cross talk between H_2S and nitric oxide. H_2S levels detected via a double antibody sandwich ELISA test were seen to be different in healthy patients, unstable angina patients, and ST elevation MI patients [109]. The patients with MI had significantly higher H_2S levels as compared to unstable angina patients, and the unstable angina patients had higher levels than healthy controls. Whether or not this new marker may help further discriminate myocardial injury in patients presenting with chest pain remains to be determined.

Panels and Point-of-Care Assays

Emerging data has shown that point-of-care (POC) testing in ACS could facilitate more rapid test results, and reduce hospital costs by reducing triage time and treatment decisions [110]. There are several POC devices on the market that have been able to measure multiple cardiac markers (mainly cTn, CK-MB, and myoglobin) in order to increase the sensitivity of myocardial damage detection. These devices require between two and six drops of blood and can usually process a result within 15 min [111]. The available products that measure POC panels in ACS as well as their US FDA and European Union CE clearance status are included in Table 1.

Ideally, these assays should meet a 10% coefficient of variation at the 99th percentile value or upper reference limit but 20% is also deemed to be acceptable [110, 112, 113]. However, not all of the previous assays comply with that recommendation, rendering some of them unacceptable for clinical use [110]. As POC testing continues to evolve, precision is only expected to improve. With a continued decrease in test turnaround time, triage time, and associated healthcare costs, these POC tests may become the future of emergency cardiac care although their sensitivity would have to improve in order to match the higher sensitivity troponin assays.

Conclusion

Over the past few decades, troponin assays have evolved to a significant level of detection sensitivity. A fifth-generation troponin assay is now cleared for use in the USA, and as more high-sensitivity assays become available, they are likely to become the standard of care for ACS diagnosis. Single measurements can be used based on the timing of presentation. Alternatively, consecutive testing may be used in combination with ECGs in order to increase sensitivity of MI detection. Algorithms utilizing these highly sensitive assays are able to classify patients into extremely low risk groups with 30-day death and mortality <1%. TMAO and other novel markers may also provide additional prognostication and risk discrimination beyond troponin assays alone. With increasing sensitivity of these markers, the specificity for cardiac pathology decreases to various levels depending on the assays used. As always, it remains critical for clinicians to use these markers judiciously and interpret results in the correct clinical context.

Compliance with Ethical Standards

Conflict of Interest   Stanley L. Hazen is named as inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. He is also a paid consultant for Esperion and P&G, and has received research funds from Astra Zeneca, P&G, Pfizer Inc., Roche Diagnostics, and Takeda. He has also received royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland HeartLab, Esperion, and Siemens. Haitham M. Ahmed declares no competing interests.

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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Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

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