Biomarkers for outcomes following acute coronary syndromes

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Abstract

Mortality attributed to acute coronary syndromes (ACS) has declined steadily since 1968. Biomarkers, such as troponin, have contributed to the observed decline by improving diagnostic sensitivity and specificity, as well as by improving patient risk stratification. Emerging biomarkers, including BNP and CRP, may have additional roles in improving risk stratification following ACS. With an improved understanding of the pathophysiology of atherothrombosis, advanced technology, and an increased ability to efficiently screen and reliably measure molecular, cellular, and other blood-borne biomarkers, the overall role of biomarkers in clinical decision making is expected to expand exponentially. To this end, biomarkers will require strict standards for development, investigation, and validation in carefully designed clinical trials before being adopted into routine patient care. Here, we review established and emerging biomarkers for assessment and management of post-ACS outcomes.

Key words: biomarker, acute coronary syndrome, infarction.

Introduction

Cardiovascular disease remains the leading cause of death worldwide. In the United States alone, an acute coronary syndrome (ACS) occurs every 26 s, resulting in death once every minute [1]. Although the rate of death attributed to ACS has declined steadily since 1968 due to improvements in both diagnosis and management, 33-43% of patients with ACS will die within five years of initial diagnosis [1]. Patients remain at substantial risk for recurrent ACS, heart failure, stroke, and sudden death.

Biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [2]". Accordingly, biomarkers have been used for diagnostic and prognostic purposes as well as to guide early management. The role for biomarkers in the diagnosis and treatment of ACS is well-established [3]. Less established, however, is the employment of biomarkers following ACS to determine long-term, progressive, or dynamic risk over time. As a result, management strategies remain oriented primarily to early risk evaluation and treatment. With advances in our understanding of the molecular underpinnings of ACS, the number of biomarkers will undoubtedly increase; however, an emphasis must be placed on translatable biomarkers with documented value in patient-specific care. Strict standards for defining

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and ultimately establishing the clinical utility of biomarkers will be an absolute prerequisite for success [4]. Here, we summarize current and evolving constructs of biomarker science as a prognostic platform among patients following ACS.

Pathophysiology of acute coronary syndromes

Acute coronary syndromes are the clinical end-result of a complex interplay of advanced atherosclerosis and thrombosis. Partial or complete occlusion of a coronary artery, not infrequently with concomitant distal embolization, at sites of plaque disruption impairs arterial flow, oxygen delivery to functioning myocytes, and results in either myocardial ischemia or infarction [5, 6].

Following myocardial infarction (MI), a highly prothrombotic local and systemic environment increases the risk for recurrent arterial thrombosis. In addition, under-perfused and injured myocytes, with or without myocardial remodeling, establish a powerful substrate for neurohumoral activation, inflammation, arrhythmia, and heart failure. An increasingly in-depth understanding of coronary atherogenesis, thrombogenesis, and post-infarction remodeling has led to the identification of numerous biomarkers that reflect, indirectly or directly, these contributing pathobiological events.

Markers of myocardial injury

Markers of myocardial injury and necrosis have long been used to identify and risk stratify patients with ACS. Markers such as creatine kinase (CK) and its MB isoenzyme (CK-MB), myoglobin, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase are known to elevate beyond normal plasma concentrations with MI, but lack tissue specificity. For example, skeletal muscle injury increases CK and CK-MB. Troponins T, C, and I are specialized components of the cardiac contractile apparatus and under normal conditions circulate in very low concentrations. Accordingly, they offer greater specificity for detecting myocardial injury than first generation cardiac biomarkers [3, 7].

Cardiac troponins T and I have been investigated extensively in the diagnosis and early management of ACS. A four-fold increase in mortality has been reported among patients with ACS and elevated cardiac troponins, both in clinical trials and routine practice registries [8, 9]. For patients with ST-segment elevation infarction (STEMI), cardiac troponins at presentation are also predictive of future mortality [10, 11]. While much of the data for predicting mortality using troponin followed outcomes for less than 6 months, troponin levels at presentation have prognostic value for up to 3 years following ACS [12]. Interestingly, although a major component of the cardiac contractile apparatus, peak troponin values during ACS may not correlate with final infarct size as well as other biomarkers such as CK-MB [13]. Therefore, the prognostic value of troponin measurements in the early stages of ACS may reflect the amount of injury upon initial presentation and identify patients at particularly high risk for future cardiac events [14]. Patients with unstable angina and non-ST-segment elevation MI (UA/NSTEMI) with elevations of troponin are known to benefit from low-molecular weight heparin (LMWH) compared to unfractionated heparin, use of glycoprotein IIb/IIIa (GP IIb/IIIa) inhibitors, and early coronary intervention [15-18].

Troponin levels also carry prognostic significance in the follow-up of patients with ACS. Eggers *et al.* followed troponin values at 6 weeks, three months, and 6 months following UA/NSTEMI in 1092 patients from the FRISC-II dataset. A persistent elevation of serum troponin I carried a hazard ratio (HR) of 1.5 (95% CI 1.1-2.0, $p = 0.01$) for 5 year mortality even after adjustment for traditional risk factors and the use of early angiography. Troponin levels were not independently associated with reinfarction after adjustment for other variables [19]. Persistent troponin elevation was associated with elevation of N-terminal pro-brain natriuretic peptide (NT-BNP) 6 months after ACS, male gender, and conservative use of angiography. Furthermore, patients with persistent troponin elevation had a lower ejection fraction. Considering the available information collectively, a persistent troponin elevation following ACS may identify patients at risk for chronic heart failure (CHF) and its complications, including sudden cardiac death [20].

Markers of thrombosis
Endothelial cell and platelet activation

The recruitment of platelets to a site of endothelial cell injury or plaque disruption with thrombus formation is the fundamental vascular phenotype of ACS. von Willebrand factor (vWF), a large glycoprotein with both hemostatic and thrombotic potential plays several contributing roles in atherothrombosis: interaction with glycoproteins Ib/V/IX and GP IIb/IIIa on circulating platelets and binding of subendothelial collagen with local release of factor VIII for participation of thrombin generation [5, 21]. Several groups have investigated serum vWF levels as a potential surrogate for endothelial cell activation. Indeed, an elevated level of vWF during the initial 48 h following ACS onset is predictive of 30-day major cardiovascular events [22-24]. vWF levels may also have a role in predicting long-term cardiovascular events. An elevation 30 days after ACS is associated with

increased risk for recurrent MI and cardiovascular death up to 10 years [25, 26].

Several groups have investigated the prognostic potential of several markers of platelet activation in patients with ACS. Activated platelets secrete CD40 ligand (CD40L), which in turn contributes to thrombosis via its interface with other platelets and inflammatory cells. Increased CD40L levels at the time of ACS are associated with a risk of death and recurrent ischemic events up to 6 months [27, 28]. However, the predictive ability of CD40L may not be independent of other biomarkers such as BNP, CRP, and troponin [29, 30]. CD40L levels may be useful, however, in identifying patients most likely to derive benefit from high dose statin therapy and from GP IIb/IIIa receptor antagonists [27, 28]. Some of the variability in study results has been ascribed to whether serum or plasma is used for CD40L measurement. Although serum CD40L tend to be systematically higher than plasma levels, the prognostic potential of serum and plasma measurements is unlikely to different [30]. Other markers of platelet activation, such as myeloid basic protein 8/14, may be useful in predicting post-ACS outcomes [31].

Markers of thrombus formation

Fibrinogen plays a key role in platelet-dependent thrombosis by linking adjacent platelets via GP IIb/IIIa receptors and by serving as the precursor for fibrin. Several studies have investigated hemostatic factor kinetics in the setting of ACS [32]. Fibrinogen levels at the time of hospital admission for ACS may provide prognostic information – particularly the risk for cardiovascular death out to 37 months [12, 33, 34]. Another marker of hemostasis that has recently been studied is thrombus precursor protein (Tpp), a type of soluble fibrin. Mega et al looked at Tpp levels in 2349 patients with ACS from the OPUS TIMI 16 trial that were treated with oral tirofiban. Even after adjusting for other clinical characteristics and biomarkers, higher Tpp levels at study inclusion were associated with an increased risk for death, MI, or urgent revascularization for up to 10 months [35].

D-dimer is a measurable fibrin degradation product. Oldgren *et al.* studied the prognostic significance of D-dimer levels among patients with ACS and found D-dimer elevation to be associated with mortality at a median follow-up of 29 months. In contrast, D-dimer levels were not associated with 30-day risk for recurrent ischemic events, suggesting that it may be a particularly robust predictor of long-term outcomes [36]. Moss *et al.* attributed a HR of 2.43 (95% CI 1.49-3.97, p = 0.0003) to D-dimer elevation two months following MI over an average follow-up of 26 months [37]. In the ESTEEM trial, of ximelegatran versus placebo for the secondary prevention of ischemic events, D-dimer measures obtained five days after initial presentation were not associated with new ischemic events at 6 months; however, an elevated level did identify patients who benefitted from treatment with a direct thrombin inhibitor [38].

Markers of inflammation

C-reactive protein

C-reactive protein (CRP) is a 25 kDa protein that is secreted by the liver as an acute phase reactant. C-reactive protein levels are increased in a wide variety of inflammatory states including infectious, rheumatologic, and malignant diseases. As the role of inflammation in atherogenesis has been recognized, the association of CRP levels with cardiovascular outcomes has been intensely studied [39, 40].

Numerous studies have been investigated CRP levels in the setting of ACS [7]. A majority were observational in nature with CRP determinations performed upon initial presentation. While the published literature reveals conflicting results, cohorts studies of relatively large sample size have demonstrated an association between raised CRP levels and ischemic-thrombotic endpoints [41, 42]. The largest study, including 7108 patients with non-ST-elevation ACS participating in GUSTO IV, showed patients with CRP levels > 1.84 mg/l had an odds ratio for death at 1 month of 1.72 (95% CI of 1.17-2.55) after adjusting for other clinical variables including troponin T. Further, CRP is an independent predictor of all- cause mortality, but not recurrent MI at 30 days [31, 41, 43-46]. Data on CRP as a prognostic biomarker following STEMI are more limited but suggest a relationship similar to other ACS [7].

The prognostic potential of CRP following ACS persists for up to 3 years [7]. The uniformity of results with longer follow-up suggests that CRP may be particularly well suited for long-term risk assessment. Ferreiros et al determined CRP levels in 105 patients with UA and found increased sensitivities for predicting 90 day outcomes with measurements at 48 h and at discharge compared to levels drawn at admission [46]. Indeed, CRP measurement may be most useful one month after ACS. In the PROVE-IT study, treatment with intensive statin therapy compared to moderate intensity statin therapy resulted in lower CRP levels at 30 days, and patients with lower CRP levels derived benefit from intensive statin therapy independent of LDL lowering [47].

Despite a respectable amount of data linking CRP, atherosclerosis and clinical events, the fundamental relationship remains unclear.

Cytokines

Cytokines are small molecules with autocrine, endocrine, and paracrine effects that have an important role in both inflammation and thrombosis. Specific cytokine responses may modulate the atherosclerotic phenotype. Cytokines related to the T_H1 response, such as interferon-γ, IL-10, and tumor necrosis factors, are associated with macrophage activation and atherogenesis. By contrast, cytokines such as TGF-β and IL-10 are associated with a T_H2 response and may be anti-atherogenic. However, in the presence of established atherosclerosis, a T_H2 response may result in aneurysm formation [6].

Several pro-atherogenic cytokines have been evaluated for association with outcomes in ACS. Pentraxin 3 is a cytokine produced in the heart in large amounts following experimental models of infarction and may have specificity for cardiovascular inflammation [48]. In a cohort of 723 patients with STEMI, admission pentraxin 3 levels were predictive of 3 months mortality independent of clinical risk factors and other biomarkers including CRP [49]. Osteoprotegrin is a soluble member of the TNF family of cytokines and increased osteoprotegrin levels have been associated with increasing atherosclerotic burden. A single study of 897 patients with acute MI attributed an adjusted hazard ratio of 1.4 (95% CI 1.2-1.7, $p < 0.0001$) to elevated circulating levels of osteoprotegrin within 24 h of admission [50]. MCP-1 is made by endothelial cells and smooth muscle cells and is important for the recruitment of macrophages. Two separate, large analyses have ascribed hazard ratios of 1.53-2.16 to increased levels of MCP-1 in patients with ACS [51, 52]. In an A to Z substudy, however, no long-term treatment benefit with high dose statin was noted despite in vitro studies suggesting otherwise [51]. While these data are encouraging, additional work needs to be done to establish a role for these cytokines in the clinical follow-up of ACS.

In addition to pro-inflammatory cytokines, levels of anti-inflammatory cytokines may have prognostic value as well. GDF-15 is a member of the TGF-β family of cytokines and is induced by ischemia and reperfusion. Reduced levels of GDF-15 are associated with increased size of infarcts in models of MI [53]. Interestingly, elevated GDF-15 levels seem to be associated with increased long-term mortality in both UA/NSTEMI patients and STEMI patients independent of other markers [54, 55]. IL-10 is the proto-typical anti-inflammatory cytokine. However, results for IL-10 are more mixed. Two small studies of patients with UA/NSTEMI associated low levels of IL-10 with increased adverse cardiac events at one to 6 months after presentation [56, 57]. By contrast, a much larger analysis of 3179 NSTEMI patients associated increased levels of IL-10 with mortality and recurrent infarction after 12 months of follow-up. Moreover, the predictive value of IL-10 was no longer significant after adjusting for CRP and IL-6 levels [58]. While GDF-15 remains a promising biomarker, the utility of IL-10 levels is more uncertain.

Inflammatory cell adhesion and activation

Following injury, epithelial cells express a number of adhesion molecules for inflammatory cells. While adhesion markers would seem to be ideal candidates for ongoing vascular inflammation, the data for their use as biomarkers is conflicting. Ray et al. looked at ICAM-1 levels in a substudy of 1164 patients with acute MI from the PROVE-IT trial. Patients in the highest quartile of soluble ICAM-1 values had an increased risk for death and recurrent ischemic events over the 2-year study period and seemed to benefit from high dose statin therapy independent of their CRP and LDL levels [59]. However, ICAM-1, VCAM-1, P-selectin, E-selectin, and L-selectin did not have predictive value for mortality after adjustment for BNP and troponin levels in a recent study of 448 patients with ACS [60].

Once cells are recruited to sites of vascular injury, they are activated to cause inflammation. Myeloperoxidase is released during the degranulation of activated macrophages and neutrophils. Three separate data sets have associated elevated myeloperoxidase levels with an increased adverse cardiac event rate at 30 days for patients with UA/NSTEMI [61-63]. However, there is conflicting data on whether these levels are still prognostic beyond 30 days [29, 62]. Importantly, interaction of myeloperoxidase levels with treatment strategies has been explored. Patients with UA/NSTEMI and an elevated myeloperoxidase level may benefit from GP IIb/IIIa inhibitors independent of troponin levels [63]. However, an association with benefit from early reperfusion is less likely [29]. Neopterin is another marker of macrophage activation. Increased neopterin levels are associated with poor outcomes in patients with STEMI and UA/NSTEMI for up to 2 years [64-66]. Patients with the highest levels of neopterin may benefit from high dose statin therapy independent of their CRP and LDL levels [66].

Neurohormonal markers
Natriuretic peptides

In response to myocyte stretch, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are secreted and stimulate natriuresis and diuresis. ANP, BNP, and BNP's precursor pro-NT BNP are reliable biomarkers for elevated left heart filling

pressures. The relation of elevated levels of natriuretic peptide levels with poor outcomes in congestive heart failure has been well described [67]. While ANP is post-translationally regulated, BNP production is regulated at the level of gene expression and can be induced by ischemia [68].

Several studies have looked at the prognostic role of natriuretic peptides in acute myocardial infarction [7]. Although the data has considerable variability in the populations studied and the cut points for which BNP was deemed to be elevated, studies have shown consistent association with both short-term mortality and long-term mortality. Reported relative risks for death with an elevated BNP for UA/NSTEMI range from 2.68-26.6 [69-76]. Less data is available for STEMI, but Khan *et al.* recently reported a HR of 3.82 (95% CI 1.89-7.78, $p < 0.001$) in patients with an increased BNP level within 24 h of the onset of chest pain. BNP levels appear to provide prognostic information independent of other clinical risk factors including troponin [74]. In addition to mortality, BNP levels at the time of ACS presentation may be predictive of future risk for heart failure [69].

Atrial natriuretic peptide levels have shown a similar ability to prognosticate outcomes following ACS. However, when adjusted for other clinical variables including BNP, ANP levels seem to add little to prognostication [77, 78].

Despite the wealth of evidence supporting the ability of BNP for risk stratification following ACS, BNP levels are routinely drawn in only about 1/5 patients in routine practice [79]. One reason for this finding may be that few interventions have been shown to reduce the heightened risk associated with an elevated BNP level. In a substudy of TACTICS-TIMI 18, which randomized patients with UA/NSTEMI to conservative management vs. an early invasive approach, no clear benefit for either strategy was identified based on BNP levels [73]. Similar results have been reported by the ICTUS study [76]. By contrast, Jernberg *et al.* suggest that BNP levels in combination with IL-6 levels may identify patients who may benefit from an early invasive approach [71]. The ongoing AVANTE-GARDE TIMI 43 trial will address the question of whether neurohormonal blockade with an angiotensin receptor blocker or a direct rennin inhibitor versus placebo will alter outcomes in patients with an elevated BNP at the time of presentation with UA/NSTEMI [80].

Adiponectin

Adiponectin is hormone produced by adipocytes with insulin sensitizing properties and has been suggested to reduce atherosclerotic burden in mouse models of atherosclerosis. Adiponectin may have direct effects on atherothrombosis by negatively

affecting platelet reactivity [81]. Indeed, low levels of adiponectin have been associated with risk for MI in an asymptomatic population [82]. Two small studies have looked at circulating adiponectin levels and outcomes up to 4 years after MI. Both show adiponectin levels to be prognostic for mortality. However, the study by Shibata *et al.* shows low levels of adiponectin to be associated with adverse cardiac events as opposed to the study by Cavusoglu *et al.*, which shows increased levels of adiponectin to predict adverse cardiac events [83, 84].

Future perspectives

The use of biomarkers for risk stratification and tailored management is one step closer to personalized medicine. Assessment of individualized risk may help to identify new candidates for therapeutics that may not have been administered otherwise. Additionally, patients can be identified who derive no apparent benefit and even harm with conventional therapeutics. One can imagine a scenario where a patient is admitted for ACS and based on markers for thrombosis, she is provided with a specific anti-platelet regimen; based on markers of injury, she is triaged between a conservative strategy versus an invasive strategy; based on neurohormonal markers, she is started on neurohormonal blockade to prevent future heart failure, and finally based on inflammatory marker levels, she is started on anti-inflammatory therapy.

The use of biomarkers to predict outcomes following ACS is, however, in its relative infancy. As outlined above, numerous candidate biomarkers have been suggested. With the completion of the human genome, numerous genome technologies have emerged that enable the entire genome, transcriptome, and metabolome in a rapid and replicable fashion. More biomarkers are bound to emerge, but how can we assimilate this data to clinical practice? Recent data suggests that panels of biomarkers may improve risk stratification much beyond more traditional risk factors [84]. As we evaluate new biomarkers, we will need to remember what makes a clinically useful biomarker. Morrow and de Lemos have outlined three criteria for a good biomarker: measurements must be rapid, reproducible, and affordable; measurements must provide information that could not otherwise be obtained via more established routes; and finally measurements should guide therapeutics [4].

The way most biomarkers are currently studied makes it difficult to achieve these goals. Most biomarker studies are done in small cohorts and in clinical trial populations designed to test different hypotheses. These trials may not be adequately powered to assess validity of novel biomarkers. In the case of clinical trials in particular, composite endpoints make specific effects of biomarkers

Table I. Summary of biomarkers associated with long-term outcomes

Marker	Risk relationship for adverse events beyond 30 days	Risk relationship for adverse events with serial evaluation	Strength of evidence for long-term outcomes
Marker of myocardial injury			
Cardiac troponin	RR of 3.1 for death [8]	HR of 1.5 for 5 year mortality [19]	A
Markers of thrombosis			
vWF	Insignificant, HR not reported [23]	30 day levels with OR of 2.41 for recurrent infarction [26]	C
sCD40L	HR 0.89-2.71 [28-30]	N/A	B
Fibrinogen	RR 2.3-4.24 [12, 33]	N/A	B
D-dimer	No HR provided but significant association [36]		C
Markers of inflammation			
CRP	RR 1.19-22.2 [7]	Levels at 30 days with RR of 1.3-1.7 for recurrent ischemic events [47]	A
MCP-1	HR 1.53-1.76 [51, 52]	Levels at 4 months with HR of 1.76 [52]	B
GDF-15	HR 1.55 [54]	N/A	C
IL-10	HR 0.43-1.7 [56, 58]	N/A	C
ICAM-1	OR 1.6 [59, 60]	N/A	C
Myelperoxidase	HR 1.3-4.7 [29, 62, 63]	N/A	B
Neopterin	HR 1.8-2.4 [65, 66]	N/A	B
Neurohormonal markers			
BNP	RR 3.6-26.6 [7]	N/A	A

OR – odds ratio, RR – relative risk, HR – hazard ratio
Level of evidence based on ACC/AHA Manual for Guideline Writing Committees: A (*Multiple population risk strata evaluated*); B (*Limited population risk strata evaluated*); C (*Very limited population risk strata evaluated*)

difficult to identify. For example, do elevated CRP levels following ACS simply predict all-cause mortality or do they predict risk for recurrent infarction? One advantage of the clinical trial setting is that interactions with treatment strategies can be identified, such as the case with CRP in the PROVE-IT trial [47]. Moreover, the statistical methodology used is important. C-statistics are useful for population based association but may not adequately represent individual risk [85]. Perhaps one approach would be to establish biorepositories for well-phenotyped patients with ACS. Novel markers could initially be tested for association with outcomes in several of these populations and tested for independence from biomarkers that are already established. Promising markers with the ability to predict additional risk beyond current methodologies could then be tested for interaction with specific interventions based on the biology of the marker. For example, promising markers of thrombosis may be tested for interaction with specific anti-platelet therapy. If a treatment interaction is identified, randomized controlled trials to validate these hypotheses can be undertaken.

In summary, biomarkers already play an important role in the diagnosis and management of ACS. Novel biomarkers are likely to emerge and may help to further reduce morbidity and mortality associated with ACS.

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