INTRODUCTION
A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, an important component or event of the pathogenic processes or pharmacologic responses to therapeutic interventions. It has multiple uses in the arenas of research and practice. In clinical practice, a biomarker may be used to diagnose a medical problem, serve as a tool for staging disease or provide an indicator of prognosis. For certain diseases, a single biomarker is enough that covers its role as defined above, while for some multifactorial diseases like coronary artery disease (CAD), the task of identifying the biomarkers, is still ongoing.

Coronary artery disease continues to be a major cause of morbidity and mortality in both men and women in developing countries. The clinical manifestation is “the acute coronary syndrome (ACS)” which encompasses unstable angina, non-ST-elevation myocardial infarction (NSTEMI) to STEMI [acute myocardial infarction (AMI)].

PATHOPHYSIOLOGY OF ACUTE CORONARY SYNDROME
The main cause of ACS is atherosclerosis in the coronary arteries. Atherosclerosis is a complex inflammatory-fibroprotective response to the retention of plasma derived atherogenic lipoproteins in the coronary artery. This deposition of lipoproteins in the coronary artery results in the formation of plaque. The first recognizable phase in plaque lesion formation is the fatty streak. It consists of mostly lipid-laden macrophages and a few T cells in the intima of medium to large elastic and muscular arteries. Fatty streaks can form anywhere, but the trend is to arise at branch points, bifurcations and curves in the arterial system. As the lesion progresses, there is migration of smooth muscle cells (SMCs) from the media to the intima and there is increase in the number of macrophages and T cells. A loose connective tissue matrix of fine collagen fibrils, elastic fibers and proteoglycans also forms. Eventually, the plaques can make the artery narrower, and less flexible making it harder for blood to flow and blood flow to the heart slows down.

In more advanced phases, the lesion is walled off from the lumen by a fibrous cap of SMCs. Small capillary-like vessels are also present, presumably to supply the thickening mass of cells with oxygen and nutrients. The expanding fibrous cap intrudes into the lumen of the artery and decreases its functional diameter. Under the fibrous cap, are trapped leukocytes and lipid that form a necrotic core. Macrophages and macrophage-derived foam cells of the atherosclerotic lesion release matrix metalloproteinases (MMPs) and other proteolytic enzymes that cause degradation of the matrix, leading to a reduction in the thickness of the fibrous cap of the lesion. When the fibrous cap ruptures, the lipid core of the atherosclerotic plaque is exposed to the blood, resulting in the recruitment of platelets, coagulation factors leading to the formation of a thrombus, causing ACS. The severe clinical manifestation of this phenomenon is AMI wherein there is complete occlusion of arteries due to the thrombus formation and sudden stoppage of blood supply to the cardiac muscles leading to irreversible muscle injury. The extent of myocardial damage after an acute coronary event of atherothrombosis determines the prognosis. Diagnosis of this ACS is based on a combination of symptoms, electrocardiographic changes and biomarkers.

The physical examination can be inadequate in differentiating atypical chest pain from chest pain of cardiac origin. On one hand 33% of patients with ACS have no chest pain. On the other hand, approximately half of the patients with acute chest pain, who have the initial diagnostic findings of ACS and are admitted to the hospital, are later found not to suffer from ACS. In the majority of patients with chest pain, the electrocardiogram (ECG) is the most readily available tool for identifying patients with ACS. However, the ECG is also often not diagnostic for acute chest pain and in fact; the sensitivity of borderline ECG for detecting ACS is only 60%.

Over the last 50 years, the contribution of laboratory medicine to the management of cardiac diseases has become increasingly sophisticated. In 1950s, Karmen et al. first reported that enzyme released from necrotic cardiac myocytes could be detected in the serum and used in the diagnosis of myocardial infarction (MI). The ensuing years witnessed progressive improvement in the type of cardiac tissue-specific biochemical markers and a corresponding enhancement in the clinical sensitivity and specificity of their routine use.

CURRENT PRACTICE OF DIAGNOSTIC BIOMARKERS IN ACS
Today markers of myocardial necrosis at the downstream of the pathophysioloogy of ACS; creatine kinase (CK) and its fraction CK-MB, myoglobin, troponins, as well as recently renin-angiotensinergic peptides have gained their mark under routine diagnosis of ACS.

Myoglobin
The main advantage of myoglobin is early detection of patients with AMI. The National Academy of Clinical Biochemistry (NACB): Laboratory Medicine Practice Guidelines (LMPG) have recommended myoglobin in addition to cardiac troponin (cTn) for the diagnosis of AMI in patients who present within 6 hours of onset.
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of symptoms. The disadvantage of using myoglobin alone is that it has poor specificity for AMI in patients with concurrent trauma or renal failure.

Creatine Kinase

The World Health Organization (WHO) proposed using the CK-MB, the specific cardiac isoenzyme for diagnosis of myocardial necrosis as well as for monitoring trends in cardiac disease. Elevation of CK-MB occurs 4–6 hours after the onset of AMI and remains for 24–48 hours. CK-MB is relatively sensitive but less specific, as it can be elevated in any condition following acute muscle injury or in patients undergoing any surgical procedure.

Cardiac Troponin

The tissue-specific troponin I and troponin T (cTnI and cTnT) detected in the peripheral circulation make them a good indicator of myocardial injury as well as of poor prognosis. The kinetics of both the troponins are such that, they are detectable in the serum within 4–12 hours after the onset of AMI and depending upon the duration of ischemia and reperfusion status, peak values occur 12–48 hours from symptom onset. Because both forms of cTnI remain in the circulation for several days after injury, it allows for diagnostic information to be available even in patients who present very late but with a disadvantage of not being able to detect reinfarction after an index event.

Natriuretic Peptides

Brain-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. BNP, an established biomarker for patients with heart failure, and NT-proBNP are elevated in ACS and can identify patients at very high risk for adverse cardiovascular events including death. The utility of BNP and NT-proBNP as markers is based on the finding that they increase in the left ventricle during remodeling after a transmural infarction or as a consequence of previous ischemic damage. However, these peptides have poor specificity for the diagnosis of ACS, since elevated levels can also be seen in patients with renal failure, primary aldosteronism, congestive heart failure and thyroid disease.

Despite the success of these biomarkers, there is still a need for the development of early markers that can reliably rule out ACS in the emergency room at presentation and also detect myocardial ischemia in the absence of reversible myocyte injury. Misdiagnosis has been reported to be the main cause of treatment delays. Undetected infarctions remain a serious public health issue and represent the leading cause of malpractice cases in the emergency settings. These imperfect strategies resulting in costly and inappropriate management decisions have forced us to search for new noninvasive quick strategies in identifying the high-risk individuals. One of them is identifying novel cardiac biomarkers.

IN SEARCH FOR NOVEL CARDIAC BIOMARKERS OF ACS

Recent investigations have been directed toward analyzing components involved in the pathogenesis of ACS, at upstream from biomarkers of necrosis, such as components released during ischemia, components of plaque destabilization and rupture, factors of thrombosis, molecules of inflammation and acute phase reactants for earlier assessment of overall patient risk of adverse event and indexing them under “biomarkers”.

Components Released during Ischemia

The explicit goal is to maintain microcirculatory flow to prevent even minor infarctions. Only marker that precedes necrosis and permits prevention of the consequence can meet the clinical need. Identifying markers of ischemia even if necrosis is not present may help in identifying a high-risk individual who may in the very near future experience the consequences of the infarct. The most promising components that have been studied in this group are free fatty acids unbound to albumin (FFAu) and choline.

Free Fatty Acids Unbound to Albumin

The observed increase in FFAu in the blood with acute myocardial ischemia is due to the increased catecholamine-induced lipid hydrolysis within the heart and reduced utilization of free fatty acids after ischemia which has been evaluated for the early identification of cardiac injury. Two groups of investigators have preliminarily studied the sensitivity of this marker at patient presentation to the emergency room and have shown that FFAu was elevated, well before other more traditional markers of cardiac necrosis, and at admission had a sensitivity of greater than 90%.

Choline

Experimental studies have demonstrated that phospholipase D enzyme-catalysed release of choline from phospholipid in blood is related to major processes of myocardial ischemia and/or necrosis. Several studies suggested that the marker might improve prognostication in patients with ACS. In a study with troponin-negative patients, choline detected high-risk unstable angina with a sensitivity and specificity of 86%. Additional studies are however needed to fully investigate the clinical significance of this marker.

Thrombotic Factors

Plaque disruption and thrombus formation in coronary arteries lead to a variable degree of luminal obstruction to the blood flow and can present clinically as unstable angina or AMI and lead to sudden death. Three major determinants of thrombotic response are (1) the presence of local thrombogenic substances, (2) the local flow disturbances and (3) the systemic thrombotic propensity. Thus, apart from the local thrombogenic potential, even systemic procoagulant status may determine the severity of the acute event of thrombosis.

Soluble CD40 Ligand

The CD40 ligand (CD40L) is a trimeric, transmembrane protein, a ligand for CD40 receptor on B cell. Both have also been found on many other cells including platelets. Soluble CD40 ligand (sCD40L) is soluble fragment generated by cleaving of the surface expressed CD40L and the main source of circulating sCD40L is platelets. The binding of CD40L enhances the inflammatory response and acts prothrombotically, leading to platelet destabilization, and inhibiting endothelial regeneration. From several clinical studies, it has consistently been reported that sCD40L is elevated in patients with ACS and that it provides prognostic information with therapeutic implications independent of established cardiac markers, e.g. cTnIs. However, preanalytical conditions are decisive for the assessment of sCD40L and may preclude routine clinical use.

Tissue Factor

Tissue factor (TF) at the upfront of the coagulation pathway plays a crucial role in initiating thrombus formation after plaque rupture in patients with ACS. Tissue factor (TF) exposed from ruptured plaque is the actual trigger but systemic procoagulant status also plays an
important role. Independent of cellular TF, blood-borne soluble TF may play a role in the propagation of thrombosis which also needs monitoring in early atherosclerotic conditions. Suefuji et al. were the first in 1997 to report the role of TF in AMI, there have been many studies conducted to determine the status of plasma TF and AMI including authors wherein increased levels of TF were observed in AMI at presentation.

**Plasminogen Activator Inhibitor 1**

Plasminogen activator inhibitor 1 (PAI-1) prevents fibrinolysis and thus accelerates thrombus formation. The evidence of increased PAI levels before first AMI attack was given by Thogersen et al. (1998). In our study, increased levels of PAI-1 levels were observed in AMI patients at presentation and were also more associated with younger AMI patients. Hamstein et al. (1985) have also reported elevated circulating concentrations of PAI-1 in young men at increased risk for recurrent infarction.

**Components Involved in Plaque Rupture**

A growing understanding of the importance of atherosclerotic plaque rupture in the pathogenesis of coronary events has led to the identification of an expanding array of markers for plaque instability.

**Myeloperoxidase**

Leukocytes play a central role in atherosclerotic plaque rupture. Myeloperoxidase (MPO) is a degranulation product, secreted by a variety of inflammatory cells, including activated neutrophils, monocytes and macrophages, such as those found in atherosclerotic plaques. It possesses proinflammatory properties and may contribute directly to tissue injury. Its systemic levels predict future cardiovascular events, independent of CD40L. Strong *in vitro* support exists for the role of neutrophil activation as an adjunct pathophysiological event in ACS that is directly different from platelet activation. Collectively the current evidence supports the need for further studies into the actual role of MPO. One of the important roles of MPO in leukocytes is to activate MMPs that bring about plaque rupture.

**Matrix Metalloproteinases**

The structural integrity of myocardial extracellular matrix (ECM) is dependent on matrix-degrading enzymes. Beside protease function and vascular effects, protease detection and quantification in peripheral blood may help detect atheromatous disease stages and aid in clinical decision-making. One such enzyme is endogenous zinc-dependent endopeptidase known as MMPs. These enzymes are regulated by tissue inhibitors of metalloproteinases (TIMPs). Matrix metalloproteinases (MMPs) may degrade myocardial ECM, leading to the development of left ventricular (LV) dilatation and heart failure and their inhibition in experimental models of AMI has been associated with reduced LV dilatation and wall stress. Elevated levels of MMP-9 and its major inhibitor TIMP-1 have been demonstrated to be associated with cardiovascular death, heart failure or both but not with reinfarction. In our study, we found that there was significant increase in circulating levels of MMP-9 as well as MMP-8 in AMI at presentation. Moreover, the increase in MMP-8 was independent of high-sensitivity C-reactive protein (hsCRP) and MMP-9. MMP-2 is also shown to be elevated post-MI and is associated with poor prognosis. In another study, we observed that serum MMP-3 levels were significantly elevated at presentation of AMI, as compared to controls, while Keely et al. have demonstrated that MMP-3 peaks at 72 hours of MI and plateau levels are associated with increase in LV volume and a lower ejection fraction, at follow-up. Amongst various MMPs, it has been suggested that MMP-9 may be of value in evaluating patients after acute coronary events.

**Cathespins**

Evidence has been obtained regarding matrix degrading enzymes cathespin S, B, K, D and L in atherosclerosis. Patients with coronary artery stenosis have demonstrated increased serum cathespin L levels than those without lesions, detectable by quantitative angiography. Increased serum cathespin S has been demonstrated in patients with atherosclerosis and diabetes while increased cathespin D in both plasma and monocytes of ACS patients. In our study, increased peripheral blood levels of cathespin B and K, with decrease in their inhibitor cystatin C at the acute phase of MI were observed. Moreover, plasma concentration of MMP-9; recently identified as a novel predictor of cardiovascular mortality in patients with CAD, and also marker for plaque destabilization and rupture, demonstrated strong positive correlation with cathespin B and negative correlation with cystatin C in this AMI group.

**Components Representing Oxidative Stress**

Oxidative stress in conjunction with inflammation is one of the important initiators of atherosclerosis. However, they also play an important role in increasing the severity of pathogenesis of ACS.

**Oxidized Low-density Lipoprotein**

Oxidized low-density lipoprotein (oxLDL) is involved in very early critical steps of atherosclerosis. OxDL as well as its antibody (oxLDL Ab) have been documented to be elevated in ACS patients including AMI and unstable angina, and were suggested to be helpful in diagnosis of ACS. Imaiz et al. (2008) have reported significantly elevated levels of plasma oxLDL in patients with new-onset type ACS than in those with worsening type ACS, suggesting it to be a marker for the development of ACS. OxLDL/beta 2-glycoprotein I (beta 2-GP1) complexes implicated in atherogenesis were also demonstrated to be associated with severe CAD and a 3.5-fold increased risk for adverse outcomes.

**Lipoprotein-Associated Phospholipase A2**

Lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as the platelet-activating factor acetylhydrolase, is a monomeric enzyme that catalyzes the hydrolysis of the sn-2 ester bond, preferentially when short acyl groups are at the sn-2 position, of oxidized phospholipids. The cascade of Lp-PLA2 activity may eventually lead to plaque destabilization, increasing the possibility of rupture and thrombosis. Confirming the same, circulating levels of secretory-associated phospholipase A2 (sPLA2) were found to increase not only in various chronic inflammatory diseases but also independently predicted clinical coronary events in patients with unstable angina and documented CAD.

**Molecules of Inflammation**

Although molecules of inflammation may have their primary role as the indicators of endothelial dysfunction and in development of atherosclerotic plaque, their soluble levels have been implicated in various studies to be associated with acute infarction.

**High-sensitivity C-reactive Protein**

The benefits of hsCRP testing in a primary setting to screen for ischemic heart disease is very clear. People are risk stratified based on the amount of CRP in blood. There are three groups: (1) less than 1 mg/L of CRP is low-risk group, (2) between 1 and 3 mg/L is classified as the moderate-risk group and (3) more than 3 mg/L is the high-risk group. At the other extreme, it is thought that one of the driving forces causing atheromatous plaques to rupture or erode, causing a cascade of events leading to coronary artery occlusion, is inflammation in the plaques. C-Reactive Protein (CRP) itself mediates atherothrombosis
which is supported by a fairly large body of evidence. CRP is elevated post-ACS, almost exclusively in the setting of myocardial necrosis indicating the level of myocardial inflammation. In a study carried out by us, we observed a three fold increase in the total hsCRP levels in MI patients at presentation, as compared to controls. One of the difficulties with CRP is that it is nonspecific and also is elevated in the presence of other inflammatory conditions (rheumatoid arthritis, malignancy, vasculitis, etc.). A new assay for human pentraxin 3 is now available. Human pentraxin 3 is an isof orm which is secreted exclusively in vascular endothelium and therefore may be more specific to the vascular plaque inflammatory activity. It remains to be seen if this biomarker can provide incremental information.

Thus, analysis of noninvasive pathobiologically diverse contributors of the progression of ACS could add complementary information in a variety of clinical settings. The role of these components in multimarker testing, in identifying the high-risk individuals, the pathophysiologic stage of the disease and tailoring therapy needs to be established. The future of ACS management would probably shift from single to multimarker testing. This will help in better characterization of each individual case by using a combination of both established and new markers for risk assessment and clinical decision-making that will substantially improve the outcomes in patients with ACS.

ACKNOWLEDGMENTS

The authors would like to acknowledge Sir HN Hospital and Research Centre and Rajawadi Municipal Hospital, Mumbai, India for recruitment of patients and Sir HN Medical Research Society for the financial support given for carrying out projects related to this topic.

BIBLIOGRAPHY