In Search for Novel Biomarkers of Acute Coronary Syndrome

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1. Introduction

Coronary artery disease (CAD) and its one of the severe clinical manifestation, Acute Myocardial Infarction (AMI) continue to be significant cause of morbidity and mortality in both men and women around the world. The extent of myocardial damage after an acute coronary event of atherothrombosis determines the prognosis. The diagnosis of acute coronary syndrome (ACS) encompassing unstable angina, non ST elevated myocardial infarction (NSTEMI) (Bertrand et al., 2002; Braunwald et al., 2000; Hamm et al., 2001) to STEMI (AMI) (Alpert et al., 2000), is based on a combination of symptoms, electrocardiographic changes and biomarkers.

The physical examination can be inadequate in identifying 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 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% (Canto et al., 2000, Panteghini, 2002).

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 could be used in the diagnosis of 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 use.

1.1 Current practice of diagnostic biomarkers in ACS

Today, markers of myocardial necrosis at the down stream of the pathophysiology of ACS; some specific to myocardial necrosis have gained their mark under routine diagnosis of ACS (Table 1).

1.1.1 Myoglobin

The main advantage of myoglobin is early detection of patients with AMI (Gibler et al., 1987, Roxin et al., 1984). The NACB Laboratory Medicine Practice Guidelines have recommended
myoglobin in addition to cardiac troponins (cTn) for the diagnosis of AMI patients who present within the 6 hours of onset of symptoms (Apple et al., 2007). The serum myoglobin level rises faster than Creatinine Kinase-MB (CK-MB) and cTn, reaching twice the normal values within 2 hours and peaking within 4 hours of symptom onset. The disadvantage of using myoglobin alone is that it has poor specificity for AMI in patients with concurrent trauma or renal failure.

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<td>Myoglobin</td>
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<td>Creatine Kinase-MB</td>
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Table 1. Current Biomarkers

1.1.2 Creatinine Kinase (CK)

CK-MB, the specific cardiac isoform of CK can be used in the diagnosis of myocardial necrosis (Mair et al., 1991). This was proposed by World Health Organization and was later extended for monitoring trends in cardiac disease (Apple et al., 2007, 2003). Elevation of CK-MB occurs 4 to 6 hours after the onset of acute MI and remains for 24 to 48 hours. CK-MB is relatively sensitive but less specific as it can be elevated in any conditions following acute muscle injury or in patients undergoing any surgical procedure. Furthermore CK is present in skeletal muscle, intestine, diaphragm, uterus and prostate and thus the specificity of CK-MB is impaired in the setting of injury to these organs. Moreover, serial analysis of CK-MB are required for quantitation as well as qualitative assessment of injury to cardiac muscle, therefore, many studies have suggested that a single cTn can be used as a convenient, cost effective and non invasive method for the diagnosis of myocardial necrosis (Apple et al., 1999, De Winter et al., 1995).

1.1.3 Cardiac troponin (cTn)s

Undisputedly troponin (cTnI and cTnT) are the most sensitive and specific biomarker of myocardial injury (Bleier et al., 1998). The kinetics of both the troponins are detectable in the serum within 4 to 12 hours after the onset of acute MI and depending upon the duration of ischemia and reperfusion status, peak values occur 12 to 48 hours from symptom onset (Apple et al., 1999). The tissue specificity and reliable detected concentration of cTn in the peripheral circulation makes it a good indicative of myocardial injury (Bleier et al., 1998). Moreover, several studies have shown that patients presenting with elevated cTns had a poor prognosis compared to those without detectable cTns (Panteghini, 2002). Because both forms of cTn remain in the circulation several days after injury, it allows for diagnosis even in patients who present very late (Apple et al., 2003). However because of long half lives, one of the disadvantages using the cTn is that neither cTnI nor cTnT can be used for detection of reinfarction after an index event. The other disadvantage of cTnT is that it is present in small amounts in skeletal muscle and is re -expressed in diseases that involve skeletal muscle degeneration. Therefore, an elevated cTn without clinical evidence of ACS should prompt for other possible myocardial injuries, including cardiac trauma, cardiac failure, and hypertension (Panteghini, 2002).
1.1.4 Natriuretic peptides

B-Natriuretic peptide (BNP) and its prohormone N-terminal pro BNP (Nt-proBNP) are neurohormones secreted from cardiac ventricles in response to ventricular wall stress (de Bold, 1985, Nakako et al., 1992). BNP, an established biomarker for patients with heart failure, and NT-pro BNP are elevated in ACS and can identify patients at very high risk for adverse cardiovascular events including death (de Lemos & Marrow, 2002, Ishibashi, 2002). The utility of BNP and NT-pro BNP as markers is based on the finding that it increases in the left ventricle during remodeling after a transmural infarction or as a consequence of previous ischemic damage (Lorgis et al., 2007). 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 new non-invasive quick strategies in identifying the high-risk individuals. One of them is identifying novel cardiac biomarkers.

Biomarkers have multiple uses in the arenas of research and practice. It is 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 interventions. 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 differential prognosis.

1.2 In search for novel cardiac biomarkers of ACS

Recent investigations have been directed towards 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, components representing oxidative stress, molecules of inflammation and acute phase reactants for earlier assessment of overall patient risk of adverse event and indexing them under “Biomarkers” (Table 2).

1.2.1 Components released during ischemia

The explicit goal is to maintain micro-circulatory 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 very near future experience the consequences of the infarct. The components that have been studied in this group are Free Fatty Acids unbound to Albumin (FFAu), Choline, Ischemia-Modified albumin (IMA) and Heart type Fatty Acid Binding Protein (H-FABP).
Components Released during Ischemia

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<td>Cystatin C</td>
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Table 2. Emerging Biomarkers

1.2.1.1 Free Fatty Acids unbound to albumin (FFAu)

Increased blood catecholamines in association with ischemia can cause increased FFA by activating lipid hydrolysis within the heart and adipose tissue. Apart from this, reduction of FFA use after ischemia can also cause increased serum concentration of FFA. The observed increase in free fatty acids unbound to albumin (FFAu) in the blood with acute myocardial ischaemia has been evaluated for the early identification of cardiac injury (Kleinfeld et al., 1996). 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 had at admission sensitivity of >90% (Kleifeld et al., 2002, Adams et al., 2002).

1.2.1.2 Ischemia Modified Albumin (IMA)

Due to ischemia the metal binding site on the amino terminus of albumin is damaged. The albumin of patient with myocardial ischemia exhibit lower metal binding capacity for cobalt than that of normal patients (Bar-Or et al., 2001a). IMA gained its importance as it demonstrated a good “negative predictive value.” In the assay, Cobalt less bound to albumin reacts with the indicator (Bar-Or et al., 2000). Significant changes in albumin cobalt binding were documented to occur minutes after transient ischemia induced by balloon angioplasty and to return to baseline within 12 hours (Bar-Or et al., 2001b). However its
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presence during ischemia of any other organ and in individual with inherent reduced cobalt binding giving false positive results, lost its specificity for routine use in detection of ischemia (Collinson & Gaze, 2008). However correlating with the clinical conditions and other markers may find its use in identifying high risk individuals.

1.2.1.3 Choline

Choline is a biomarker that is released when phospholipids are cleaved, which suggests that perhaps it could be a marker of ischemia and/or necrosis (Danne & Möckel, 2010). Experimental studies have demonstrated that phospholipase D enzyme activation and release of choline in blood are related to major processes of myocardial ischemia. Several studies suggested that the marker might improve prognostication in patients with ACS (Danne et al., 2003). 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 (Apple et al., 2005).

1.2.1.4 Heart type Fatty Acid Binding Protein (H-FABP)

H-FABP is a low molecular weight protein involved in myocardial fatty-acid metabolism (Glatz et al., 1997). This protein is rapidly released immediately after infarction. H-FABP has been shown in mouse studies to be an early marker of ischaemia (Glatz et al., 1988) (before morphological evidence of myocardial necrosis) and can therefore help with diagnosis of MI earlier (Glatz et al., 1988, C. P. Chen et al., 2004, L. Chen et al., 2004). However, studies attempting to use H-FABP alone for early diagnosis of AMI have produced disappointing results. One review of six studies found that the pooled positive predictive value to be 65.8% and pooled negative predictive value to be 82.0% (Body, 2009). Also it still lacks cardiac specificity as it is found in brain, kidney and skeletal tissue and levels can go up in acute ischaemic strokes and intense exercise. Thus its role as biomarker needs further evaluation.

In a recent study, Bhardwaj et al (2011) evaluated an array of established and emerging cardiac biomarkers for ACS among patients with chest discomfort in the emergency department. In their study neither IMA nor H-FABP detected or excluded ACS. Among patients with symptoms suggestive of ACS, results for NT-proBNP, hsTnI or FFA added diagnostic information to cTnT. In the context of hsTnI results, FFA measurement significantly reclassified both false negatives and false positives.

1.2.2 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 a sudden death. Three major determinants of thrombotic response are (a) the presence of local thrombogenic substances, (b) the local flow disturbances and, (c) the systemic thrombotic propensity. Thus apart from the local thrombogenic potential even systemic pro-coagulant status may determine the severity of the acute event of thrombosis.

1.2.2.1 sCD40 ligand (sCD40L)

The CD40 and CD40 ligand (CD40L) system is expressed on a variety of cell types including activated platelets, vascular endothelial cells, vascular smooth muscle cells, monocytes, and macrophages. CD40L is a trimeric, transmembrane protein (Hennet et al., 2001). Following expression on the cell surface, CD40L is partly cleaved by proteases and subsequently
released into the circulation as sCD40L which can be detected in serum and plasma. The main source of circulating sCD40L is platelets (Hennet al., 2001). The binding of CD40L enhances the inflammatory response, acts prothrombotically, leads to plaque destabilization, and inhibits 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. cardiac troponins (Heesechen et al., 2003). However, pre-analytical conditions are decisive for the assessment of sCD40L and may preclude routine clinical use (Weber et al., 2006).

1.2.2.2 P-selectin

P-selectin is a cell surface glycoprotein that plays a critical role in the migration of lymphocytes into tissues. It is found constitutively in a preformed state in the Weibel-Palade bodies of endothelial cells and in α-granules of platelets. This stored P-selectin is mobilized to the cell surface within minutes in response to a variety of inflammatory and thrombogenic agents. The mobilized P-selectin is apparently present on the cell surface for only a few minutes after which it is recycled to intracellular space. P-selectin also binds monocytes and neutrophils in addition to activated platelets and is responsible for incorporation of leukocytes into the growing thrombus (Malý et al., 2003). Thus, P-selectin is a marker of platelet activation which in turn is prerequisite for thrombosis (Serebruany et al., 1999a). Fijnheer et al (1997) have concluded that endothelial cell activation is associated with an increased P-selectin concentration per platelet. Elevated levels have been reported not only in AMI and unstable angina but also in stable angina. In our study significant negative correlation of sP-selectin with age in AMI group suggests increased pro-coagulant status in younger AMI patients (Mashru et al., 2010). Its role as biomarker requires extensive clinical evaluations.

1.2.2.3 Tissue Factor (TF)

TF at the upfront of the coagulation pathway plays a crucial role in initiating thrombus formation after plaque rupture in patients with ACS. Tissue injury disrupts vascular endothelium causing its release into circulating blood and hence activation of coagulation cascades. It activates extrinsic pathway of coagulation and act as cofactor for Factor VII (fVII) and initiates cell surface procoagulant activity. It is also known to activate factor X through intrinsic pathway by activating factor IX, leading to thrombin generation and fibrin formation. Since Suefuji et al in 1997 reported the role of TF in AMI, there have been many studies conducted to determine the status of plasma TF and AMI (Kamikura et al., 1997, Nishiyama et al., 1998, Malý et al., 2003, Morange et al., 2007, Xiong et al., 2007) with contradictory findings. We observed increased levels of TF in AMI at presentation (Shalia et al., 2010a). TF exposed from ruptured plaque is the actual trigger but systemic procoagulant status also plays 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.

1.2.2.4 Plasminogen Activator Inhibitor-1 (PAI-1)

PAI-1 prevents fibrinolysis and thus accelerates thrombus formation. Immunohistiochemical staining of coronary artery specimens (Shindo et al., 2001) and mRNA expression studies (F. Chen et al., 2005) have demonstrated increased expression of PAI. While 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 were observed in AMI patients at presentation and were also more associated with younger AMI patients (Shalia et al., 2010). Hamstein et al (1985) have also reported elevated circulating concentrations of PAI-1 in young men at increased risk for recurrent infarction.

1.2.3 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 rupture. The enzymes that have gained importance in this aspect are myeloperoxidase, matrix metalloproteinases, cathepsins, etc.

1.2.3.1 Myeloperoxidase (MPO)

Leucocytes play a central role in atherosclerotic plaque rupture (de Servi et al., 1996). Myeloperoxidase 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 (Eiserich et al., 2002). Its systemic levels predicted future cardiovascular event independent of CD40L (Baldus et al., 2003) and gave in-vitro strong support to 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 Myeloperoxidase in leucocytes is to activate metalloproteinases that bring about plaque rupture (Zhang et al., 2001).

1.2.3.2 Metalloproteinases (MMPs)

The structural integrity of myocardial Extracellular Matrix (ECM) is dependent on endogenous zinc-dependent endopeptidases known as matrix metalloproteinases (MMP). These enzymes are regulated by tissue inhibitors of metalloproteinases (TIMPs) (Kelly et al., 2008). MMPs may degrade myocardial ECM leading to the development of 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 TIMP1 have been demonstrated to be associated with cardiovascular death, heart failure or both but not with re-infarction (Kelly et al., 2007). 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 sensitive C-reactive protein (HsCRP) and MMP-9 (Shah et al., 2009). MMP-2 is also shown to be elevated post MI (Dhillon et al., 2009) and is associated with poor prognosis (Kelly et al., 2008a). In another study we observed that Serum MMP-3 levels were significantly elevated at presentation of the acute MI as compared to controls (Shalia et al., 2010b) while Kelly et al (2008b) 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 (Apple et al, 2005).

1.2.3.3 Cathepsins

Evidence has been obtained for expression in human atherosclerotic lesion of another matrix degrading enzymes cathepsin S, B, K, D and L (Jormsjö et al., 2002). Beside protease function
and vascular effects, protease detection and quantization in peripheral blood may help detect atheromatous disease stages and aid in clinical decision-making (Vivanco et al., 2005). Patients with coronary artery stenosis have demonstrated increased serum cathepsin L levels than those without lesions detectable by quantitative angiography (Liu et al., 2006a). Increased serum cathepsin S has been demonstrated in patients with atherosclerosis and diabetes (Liu et al., 2006b) and increased cathepsin D both in plasma and monocytes of ACS patients. In our study increased peripheral blood levels of cathepsin B and K and decrease in their inhibitor cystatin C at the acute phase of MI were observed (Shalia et al., 2011). 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 cathepsin B and negative correlation with cystatin C in AMI group (Shalia et al., 2011).

1.2.3.4 Pregnancy-Associated Plasma Protein-A (PAPP-A)

It is known as high molecular weight (200kDa) glycoprotein synthesized by the syncytiotrophoblast and is typically measured during pregnancy for Down syndrome screening. It is pro-atherosclerotic molecule family of proteins; a member of the insulin-like growth factor (IGF) -dependent IGF binding protein-4 specific metalloproteinase (Bayes-Genis et al., 2000). It is thought to be released when neovascularization occurs and thus may be a marker of incipient plaque rupture which was later confirmed in studies demonstrating its increased expression in unstable plaques and their extracellular matrices (Bayes-Genis et al., 2001) and corresponding increased circulating levels in unstable angina and AMI (Bayes-Genis et al., 2001). Interestingly, it demonstrated increase in risk of cardiovascular death, MI or revascularisation even without a raised Troponin (Lund et al., 2003). Evidence for the use of this biomarker clinically remains scarce but promising. More studies and standardized assays will be needed to improve its clinical utility.

1.2.4 Components representing oxidative stress

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

1.2.4.1 Oxidized LDL (Ox-LDL)

Ox LDL is involved at very early critical steps of atherosclerosis. Oxidized LDL as well as its antibody (ox-LDL 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 (Zhou et al., 2006). Imazu et al (2008) examined the relationship among plasma levels of OxLDL, measured by an enzyme immunoassay using an antibody against OxLDL (FOH1a/DLH3) and apolipoprotein B, at the onset of ACS. Plasma levels of OxLDL were significantly higher in patients with new-onset type ACS than in those with worsening type ACS (2.98 versus 1.53 mg/dL, P = 0.002). In conclusion, plasma levels of OxLDL were demonstrated to be associated with CHD and significantly higher in patients with new-onset ACS. The findings of the study suggested that plasma OxLDL can be a marker of the development of ACS. Oxidized low-density lipoprotein (oxLDL)/beta(2)-glycoprotein I (beta2GPI) complexes implicated in atherogenesis were also demonstrated to be associated with severe coronary artery disease and a 3.5-fold increased risk for adverse outcomes (Greco et al., 2010).
1.2.4.2 Lectin-like Oxidized low density lipoprotein receptor-1 (LOX-1)

LOX-1 is a multi-ligand receptor, whose repertoire of ligands includes oxidized low-density lipoprotein, advanced glycation endproducts, platelets, neutrophils, apoptotic/aged cells and bacteria. Sustained expression of LOX-1 by critical target cells, including endothelial cells, smooth muscle cells and macrophages in proximity to these ligands, sets the stage for chronic cellular activation and tissue damage suggesting the interaction of cellular LOX-1 with its ligands to contribute to the formation and development of atherosclerotic plaques. (Navarra et al, 2010). It was demonstrated to be elevated in ACS patients but not correlating with troponins or CK suggesting it not to be a marker of cardiac injury (Hayashida 2005). Kamezaki (2009) in another study have shown it to be positively correlating with urinary 8-isoprostane and negatively correlating with superoxide dismutase in ACS patients suggesting that increased serum LOX-1 reflect enhanced oxidative stress in vascular wall.

1.2.4.3 Lipoprotein-associated Phospholipase A-2 (Lp-PLA-2)

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 (Hsieh et al., 2000). Confirming the same, circulating levels of 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 coronary artery disease (Kugiyama et al., 1999, 2000).

1.2.5 Molecules of inflammation

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

1.2.5.1 Vascular Cell Adhesion Molecule (VCAM-1)

VCAM-1 is not routinely expressed under physiological conditions. Expression of VCAM-1 occurs only on activated endothelium and vascular smooth muscle cells in developing atherosclerotic lesion (Braun et al., 1999). It was demonstrated to be expressed especially in the intimai neovascularure and largely associated with leukocyte accumulation; promoting the binding of lymphocytes and monocytes which further move by diapedesis which release cytokines and enzymes important for progression of lesion as well as rupture of the plaque (O’Brien et al., 1993, 1996). Literature reports correlation of sVCAM-1 with the extent of coronary atherosclerosis with elevated levels in AMI (Bossowska et al., 2003, G ö ray ö, Erbay et al., 2004). Consistent with this finding we have observed highest levels with AMI patients and unstable angina in decreasing order as compared to controls (Mashru et al., 2010). In our study age matched analysis also demonstrated younger age group (<=40 years) of patients with AMI with highest sVCAM-1 as compared to age matched controls and in unstable angina it was more associated with females than male patients. Above observations suggest VCAM-1 to be also as an indicator towards ACS in patients with low-risk profile for cardiovascular risk factors such as age and gender.
1.2.5.2 Platelet Endothelial Cell Adhesion Molecule (PECAM-1)

PECAM-1 of the immunoglobulin superfamily is with wide variety of functions such as platelet activation, inflammation, cell survival, in the immune response and in transendothelial migration of monocytes (TEM) (Muller et al., 1993). It has also been demonstrated to have role in plaque formation and thrombosis (Newman et al., 1990, Mahooti et al., 2000). In our study (Mashru et al., 2010) there was significant increase in sPECAM-1 in AMI patients at acute event consistent with the finding of Serebruany et al (1999b) and Soeki et al (2003).

1.2.5.3 Monocyte Chemoattractant Protein-1 (MCP-1)

MCP-1 is a chemokine responsible for the recruitment of monocytes to sites of inflammation that appears to play a critical role in the promotion of plaque instability (Szmitko et al., 2003). In case control studies, plasma MCP-1 concentrations have been shown to be associated with restenosis after coronary angioplasty (Cipollone et al., 2001). However, in a prospective study on a large cohort of ACS patients, the distribution of MCP-1 values in the healthy subjects and the study population overlapped considerably. However, subsequent studies have shown that MCP-1 plasma concentration is associated with different cardiovascular risk factors, and a greater risk of developing a cardiovascular event in the future (de Lemos et al., 2003, Deo et al., 2004).

1.2.5.4 Cystatin C

This biomarker is a low-molecular-weight basic protein (13 kDa) that is freely filtered and metabolized after tubular reabsorption. It seems that it is less influenced by age, gender, and muscle mass than serum creatinine. There is a U.S. Food and Drug Administration-cleared assay that is analytically robust. Some studies suggest that it is useful for prognostication in heart failure (Sarnak et al., 2005, Shlipak et al., 2005) and ACS (Jernberg et al., 2004). This would make sense as it is well accepted that renal function is a critical determinant of prognosis. In our study cystatin C levels did not deviate much from the controls maintaining its normal levels with normal kidney functioning and demonstrated negative correlation with MMP-9 in AMI group (Shalia et al., 2011).

1.2.5.5 Interleukin 6 (IL-6)

As the prototypical acute phase reactants, interleukin-6 (IL-6) has been the focus of investigations for the diagnosis of ACS. The Fragmin and Fast Revascularisation During Instability in Coronary Artery disease II trial (FRISC) study group showed that the circulating level of IL-6 is a strong independent marker of increased mortality among patients with unstable angina and is useful in directing subsequent care. However, the best timing for measurement of IL-6 for diagnosis and risk stratification of ACS remains uncertain (Lindmark et al., 2001). Alwi et al., (2008) concluded that the IL-6 level in ACS were higher than those in CHD. The IL-6 level 4.43 pg/mL could differentiate the acute condition (ACS) and stable condition (non-ACS) with sensitivity of 89.95% and specificity of 77.42%, and ROC of 0.87.

A study by Kavsak et al (2007) demonstrated that IL-6, MCP-1, and a known biomarker, NT-proBNP were independent predictors of long-term risk of death or HF, highlighting the importance of identifying leukocyte activation and recruitment in ACS patients.
1.2.5.6 IL-10

IL-10 is an important anti-inflammatory molecule with so far contradictory findings in ACS patients. On one hand it was shown to demonstrate more favorable prognosis in patients with ACS (Heeschen et al., 2003) while on the other hand it reflected a proinflammatory state in patients with ACS which suggested that IL-10 is as effective biomarker for the risk prediction of future cardiovascular events as other markers of systemic inflammation (Mälarstig et al., 2008). Extensive study may be required to establish its role in the pathogenesis of ACS and its utility as biomarker.

1.2.5.7 IL-18

It is a member of the IL-1 cytokine family, originally identified in macrophages and Kupffer cells as factor able to induce IFN-γ production by T cells which itself is a central proatherogenic factor. Both increased serum levels of IL-18 and reduced concentrations of IL-10 have been shown to have prognostic significance in ACS. Chalikias, et al (2005) sought to assess whether the ratio of serum IL-18/IL-10 levels has higher positive predictive value than the individual measurement of IL-10 and IL-18 in patients admitted to hospital with ACS. Their findings demonstrated that significantly higher odd ratios were found for IL-18/IL-10 ratio (1.74 95% CI 1.09-2.78) compared to individual IL-18 (1.46 95% CI 0.93-2.27) and 1/IL-10 (1.63 95% CI 1.04-2.56) measurements. Recently Hartford et al (2010) demonstrated that IL-18 levels were significantly related to all-cause mortality, even after adjustment for clinical confounders (hazard ratio [HR], 1.19; 95% confidence interval, 1.07 to 1.33; P=0.002). Long-term, cardiovascular mortality was univariately related to IL-18, and the adjusted relation between noncardiovascular mortality and IL-18 was highly significant (HR, 1.36; 95% confidence interval, 1.11 to 1.67; P=0.003). IL-18 independently predicted congestive heart failure, MI, and cardiovascular death/congestive heart failure/MI in both the short and long term. Measurements from day 1 of ACS and 3 months after ACS had a similar power to predict late outcome.

The data from the PRIME Study, a prospective cohort of 9758 asymptomatic middle-aged men recruited in Northern Ireland and France between 1991 and 1993 demonstrated that higher circulating levels of Hs-CRP, intercellular CAM-1 (ICAM-1), IL6 and IL18 to be equally predictive of stable angina and ACS (all P-values of OR comparison >0.05) (Empana et al., 2008).

1.2.5.8 High-sensitivity C-Reactive Protein (HsCRP)

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 (Ridker, 2003, Shishehbor et al., 2003). CRP itself mediates atherothrombosis which is supported by a fairly large body of evidence (Pasceri et al., 2000, Nakajima et al., 2002, Nakagomi et al., 2000, Verma et al., 2002, Devaraj et al., 2003). The benefits of HsCRP testing in a primary setting to screen for ischaemic heart disease is very clear. People are risk stratified based on amount of CRP in blood. There are three groups; less than 1mg/l of CRP is low risk group, between 1 – 3mg/l is classified as the moderate risk group and more than 3mg/l is the high risk group (AHA/CDC, 2003). However, its use post-ACS or -MI is less clear. CRP is elevated post-acute coronary syndrome 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 (Shalia et al., 2011).
Elevated peak CRP in the early phase of MI is related to early mechanical complications, including cardiac rupture, ventricular aneurysm and thrombus formation (Anzai et al., 1997). CRP levels post-MI peak at two to four days, then take 8 to 12 weeks to subside to baseline levels. One of the difficulties with CRP is that it is non-specific 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 isoform which is secreted exclusively in vascular endothelium and therefore may be more specific to the vascular plaque inflammatory activity (Matsui et al., 2010). It remains to be seen if this biomarker can provide incremental information.

2. Conclusion

Thus, non-invasive indicators of separate pathobiologically diverse contributors to the progression of ACS, such as molecules of inflammation, components of plaque rupture and thrombosis, could add complementary information in variety of clinical settings. The role of these components in multi-marker 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 multi-marker testing leading to 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.

Growing hand in hand with our contemporary fascination are the promises of personalized medicine, the discovery of novel biomarkers in cardiovascular diseases which has been embraced as a major objective of government, private and industry supported research initiatives. More than a decade of advances in our understanding of the complex mechanisms underlying the initiation, progression of atherothrombosis and its complications has stimulated efforts to identify and characterize new markers associated with this processes. In addition newer screening based discovery techniques such as metalobomics and proteomics have revealed large numbers of candidate metabolites and proteins associated with this disease for which the function or role in pathophysiology has yet to be explained. The clinical application of cardiac biomarkers in ACS is no longer limited to establishing or refuting the diagnosis of myocardial necrosis. Cardiac biomarkers provide a convenient and non-invasive means to gain insights into the underlying causes and consequences of ACS that mediate the risk of recurrent events and may be targets for specific therapeutic interventions.

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4. References


Braunwald, E.; Antman, EM.; Beasley, JW.; Califf, RM.; Cheitlin, MD.; Hochman, JS.; et al. (2000). ACC/AHA guidelines for the management of patients with unstable angina and non-STsegment elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (Committee on the management of patients with unstable angina). *J Am Coll Cardiol*; Vol. 36, No. 3, (September 2000), pp. 970–1062.

Canto, JG.; Shlipak, MG.; Rogers, WJ.; Malmgren, JA.; Frederick, PD.; Lambrew, CT. et al. (2000). Prevalence, clinical characteristics, and mortality among patients with myocardial infarction presenting without chest pain. *JAMA* Vol. 283, No. 24, (Jun 2000), pp. 3223-3229.
Chalikias, GK.; Tziakas, DN.; Kaski, JC.; Hatzinikolaou, EL; Stakos, DA.; Tentes, IK.; et al. (2005) Interleukin-18: interleukin-10 ratio and in-hospital adverse events in patients with acute coronary syndrome. *Atherosclerosis*, Vol. 182, No. 1, (September 2005), pp.135-143.


Deo, R.; Khera.; A, McGuire.; DK, Murphy SA.; Meo Neto Jde, P.; Morrow, DA.; et al, (2004). Association among plasma levels of monocyte chemoattractant protein-1,


Kleinfeld, AM.; Kleinfeld, KJ. & Adams JE. (2002). Serum levels of unbound free fatty acids reveal high sensitivity for early detection of acute myocardial infarction in patient samples from the TIMI II trial. J. Am. Coll. Cardiol, Vol. 39, pp. 312A.


Mashru, MR.; Shah, VK.; Soneji, SL.; Loya, YS.; Vasvani, JB.; Shalia, KK.; et al. (2010). Soluble Levels of Cell Adhesion Molecules (CAMs) in Coronary Artery Disease. Ind Heart J, Vol. 62, No. 1, (Jan-Feb 2010), pp. 57-63.


Shalia, KK.; Shah, VK.; Mashru, MR.; Soneji, SL.; Vasvani, JB.; Payannavar, S.; et al. (2010). Matrix metalloproteinase-3 (MMP-3) -1612 5A/6A promoter polymorphism in

Shalia, KK.; Mashru, MR.; Shah, VK.; Soneji, SL. & Payannavar, S. (2011). Cathepsins and Coronary Artery Disease. Accepted in Indian Heart Journal (2011).


Atherothrombosis has reached pandemic proportions worldwide. It is the underlying condition that results in events leading to myocardial infarction, ischemic stroke and vascular death. As such, it is the leading cause of death worldwide manifested mainly as cardiovascular/cerebrovascular death. The complex and intimate relationship between atherothrombosis and traditional and novel risk factors is discussed in the following chapters of Traditional and Novel Risk Factors in Atherothrombosis - from basic science to clinical and therapeutic concerns. Beginning with pathology and pathophysiology of atherothrombosis, plaque rupture/disruption, this book continues with molecular, biochemical, inflammatory, cellular aspects and finally analyzes several aspects of clinical pharmacology.

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