Biomarkers In Cardiology

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ABSTRACT

Atherosclerosis, Myocardial ischemia, Myocardial infarction, Heart failure may be a series of events manifesting in a single patient. An increasing number of enzymes, hormones, biologic substances, and other markers of myocytic injury, which are referred as biomarkers, are being used for diagnosis and management. The biomarkers like CK- MB, CRP have become the part and parcel of today's management. The search for new ideal biomarker, which fulfills all the above criteria and has equally good sensitivity and specificity, is on. The present article has taken a review of various biomarkers used in cardiology.

Introduction

Cardiac diseases are ever increasing in today's world. Atherosclerosis, Myocardial ischemia, Myocardial infarction, Heart failure may be a series of events manifesting in a single patient. The assessment of each of these can be time consuming diagnostic challenge. Various methods of evaluation of patient right from history, ECG, to the various specialized investigations are used to arrive at a diagnosis. An increasing number of enzymes, hormones, biologic substances, and other markers of myocytic injury, which are referred as biomarkers, are being used for diagnosis and management. The biomarkers like CK- MB, CRP have become the part and parcel of today's management. An ideal biomarker would be one that is readily available to the clinician, the results of which are reproducible and gives added information not available with clinical examination or imaging modality and helps in management decision making. The search for new ideal biomarker, which fulfills all the above criteria and has equally good sensitivity and specificity, is on.

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There is no specific categorization of biomarkers but they can be divided according to their use in specific clinical syndrome as Biomarkers of Cardiac ischaemia, Heart Failure and Atherosclerosis.

BIOMARKERS OF CARDIAC ISCHAEMIA

Over the years more specific biomarkers of myocardial necrosis have become available, the accuracy of detecting myocardial infarction has changed. Currently, more specific, and sensitive biomarkers and imaging methods to detect myocardial infarction are further refinements in this evolution.[1]

TROPONINS — The preferred biomarker for myocardial necrosis is cardiac troponin (I or T), which has nearly absolute myocardial tissue specificity as well as high clinical sensitivity, thereby reflecting even microscopic zones of myocardial necrosis.[6] Cardiac troponin I (cTnI) and T (cTnT) are cardiac regulatory proteins that control the calcium-mediated interaction of actin and myosin. Early troponin release during MI comes from what is termed as cytosolic pool. Subsequent release is prolonged with degradation of actin and myosin filaments in the area of damage.[2] It appears in serum

after 4 hours after onset of symptoms and peak at 12-48 hrs and lasts for several days to 2 weeks.[3]

These proteins are products of specific genes and therefore have the potential to be unique for the heart . Myocardial cell death can be recognized by the appearance in the blood of different proteins released into the circulation from the damaged myocytes. Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers such as cardiac troponin or CKMB are increased in the clinical setting of acute myocardial ischemia.[4] Although elevations in these biomarkers reflect myocardial necrosis, they do not indicate its mechanism. Thus, an elevated value of cardiac troponin in the absence of clinical evidence of ischemia should prompt a search for other etiologies of myocardial necrosis, such as myocarditis, aortic dissection, pulmonary embolism, congestive heart failure, renal failure.[5]

Blood samples for the measurement of troponin should be drawn on first assessment and 6–9 h later. To establish the diagnosis of myocardial infarction, one elevated value above the decision level is required. Detection of a rise and/or fall of the measurements improves the sensitivity of troponins for the diagnosis of acute myocardial infarction.[4] .The demonstration of a rising and/or falling pattern is needed to distinguish background elevated troponin levels, e.g. patients with chronic renal failure, from elevations in the same patients which are indicative of myocardial infarction.[7] However, this pattern is not absolutely required to make the diagnosis of myocardial infarction if the patient presents >24 h after the onset of symptoms. Troponin values may remain elevated for 7–14 days following the onset of infarction.[1]

In addition to diagnosing myocardial ischemia troponins were also predictors of cardiac events and severity of disease. In a study it was found that event rates in patients with negative tests were only 1.1% for Trop-T, and 0.3 % for Trop-I.[8].An elevated troponin T level is an excellent marker for ruptured plaques and severe obstructive coronary artery disease but a normal troponin T level does not imply normal coronary arteries.

Recently sensitive cardiac troponin assays have become available with an excellent diagnostic performance as early as at the time of a patient's presentation in the emergency department and may thereby substantially improve the early diagnosis of acute myocardial infarction, particularly in patients with a recent onset of chest pain.[36]

CK-MB

This enzyme is expressed in many body tissues and catalyses the conversion of creatine to phosphocreatine .The enzyme has three different isoenzymes with different tissue distributions. The CK-MB isoenzyme is present in highest concentrations within the myocardium, but it is not completely specific for myocardial tissue, and constitutes 1-3% of the CK in skeletal muscles, and is present in minor quantities in intestine, diaphragm, uterus and prostrate. Therefore the specificity of CK-MB may be impaired in the setting of injury to these organs.

In patients presenting with MI the concentrations of the enzyme begins to rise 4-6 hrs after the onset of chest pain ,peak at 12-24 hrs and return to the base line within 48-72 hrs.[3,17].For the diagnosis of MI 2 consecutive samples should be elevated .A rise or fall of CK-MB provides additional evidence supporting the diagnosis of MI.

CK-MB is also useful in risk stratification.In patients with NSTEMI the 30 day mortality risk increased with CK-MB levels, from 1.8% to 3.3% to 8.3% for normal,1-2 fold increase, to 10 fold increase CK-MB levels increase respectively.

The older markers of ischemia like Aspartate

aminotransferase (AST), Lactate Dehydrogenase (LDH), Myoglobin are no longer used routinely as their specificity is low.

Among all the markers the troponins, because of highest specificity, is the preferred biomarker for risk assessment in patients with ACS. Trop-I and Trop-T appear to have similar value for risk assessment in ACS[18] The only limitation of standard cardiac troponin assay is their low sensitivity with early presentation, owing to delayed increase in circulating levels of cardiac troponins.

The currently available markers are useful after the occurrence of cardiac event.Recent biomarkers are being developed which can be markers of plaque instability or plaque rupture and may help identify patients before manifestation of ACS.

SOLUBLE CD 40 LIGAND (SCD40L)- SCD40L ligand is expressed on platelets and released from them on activation. Soluble CD40 ligand is a powerful biochemical marker of inflammatory thrombotic activity in patients with acute coronary syndromes. Soluble CD40 ligand not only contributes importantly to the pathophysiology of acute coronary syndromes but also represents a reliable and powerful clinical marker for use in identifying patients with high-risk atherosclerotic lesions, coronary thrombosis, or both [34]

MYELOPEROXIDASE

Myeloperoxidase is an excellent candidate for the prediction of acute coronary syndromes because it is released by activated leukocytes, is elevated and catalytically active in vulnerable plaques. A single initial measurement of plasma myeloperoxidase independently predicts the early risk of myocardial infarction, as well as the risk of major adverse cardiac events in the ensuing 30-day and 6-month periods. Myeloperoxidase levels, in contrast to troponin T, creatine kinase MB isoform, and C-reactive protein levels, identified patients at risk for cardiac events in the absence of myocardial necrosis, highlighting its potential usefulness for risk stratification among patients who present with chest pain.[33]

Heart fatty acid binding protein(h-FABP)

FABPs are the major vehicle for cytosolic transpost of long chain unesterified fatty acids. H- FABP appears in the blood soon after the onset of infarction, so it has been proposed as an early marker of the MI diagnosis. Its plasma concentration increases within 2-3 hours after MI and returns to normal range within 12-24 hours.In view of the fact that h-FABP rapidly returns to normal within 24 hours after MI,it can be used to assess recurrent infarction within 10 hours after first MI.The rapid release of h-FABP can also be used for detection of successful coronary reperfusion in patients with MI

ISCHAEMIA MODIFIED ALBUMIN

Ischemia modified albumin (IMA), measured by the albumin cobalt binding test (ACB), has been shown to be a marker of myocardial ischemia.. Under physiological conditions, cobalt can bind tightly to the exposed N-terminus of albumin. In the presence of myocardial ischemia, structural changes take place in the N-terminus of the protein, which reduces its binding capacity.IMA has been found to rise early and consistently in patients who develop chest pain and ST segment changes during PCI. [30]There was a significant relationship between Troponin-T and IMA, suggesting that both these biomarkers added significant information about the presence of ACS and may be useful for patients who present to the emergency room with chest pain. Serum IMA was also increased in a small proportion of patients with symptoms of stroke, suggesting that it should be considered a marker of acute ischemic events and not specific for cardiac ischaemia. [31]

BIOMARKERS IN HEART FAILURE

Natriuretic Peptides BNP and N-terminal pro–brain natriuretic peptide (NT-pro-BNP) are the peptides that are synthesized in the myocytes and released during hemodynamic stress — that is, when the ventricles are dilated, hypertrophic, or subject to increased wall tension.

Prohormone BNP is cleaved by a circulating endoprotease, termed corin, into two polypeptides: the inactive NT-pro-BNP, 76 amino acids in length, and BNP, a bioactive peptide 32 amino acids in length. BNP causes arterial vasodilation, diuresis, and natriuresis, and reduces the activities of the renin–angiotensin–aldosterone system and the sympathetic nervous system. [19]. Apart from heart failure these peptides may become elevated from other causes like Renal failure, increasing age, pulmonary hypertension

Measuring levels of BNP is most useful in the evaluation of patients with dyspnea presenting to the emergency department, with possible heart failure, when the diagnosis and management can be facilitated by knowledge of natriuretic peptide levels.[20] BNP level is also an accurate predictor of survival in patients with acute decompensated heart failure, irrespective of left ventricular ejection fraction. In the Acute Decompensated Heart Failure (ADHERE) registry, after adjustment for baseline variables, an almost linear relation between BNP level and in-hospital mortality was found.[21]

Comparing BNP and NT-pro-BNP,it was found that the N-terminal prohormone was slightly superior to BNP for predicting death or rehospitalization for heart failure. The longer half-life of NT-pro-BNP may make it a more accurate index of ventricular stress and therefore a better predictor of prognosis.[15]

TNF-α & Interleukins 1,6 & 18

In 1990, Levine et al. described elevated levels of circulating TNF- in patients with heart failure.[10]TNF-α and at least three interleukins (interleukins 1, 6, and 18) are considered to be proinflammatory cytokines and are produced by nucleated cells in the heart. Proinflammatory cytokines appear to cause myocyte apoptosis and necrosis; interleukin-6 induces a hypertrophic response in myocytes, whereas TNF causes left ventricular dilatation, apparently through activation of matrix metalloproteinases[11]

Fas (also termed APO-1)

It is a member of the TNF-α receptor family that is expressed on a variety of cells, including myocytes. When Fas is activated by the Fas ligand it mediates apoptosis and plays an important role in the development and progression of heart failure. Elevated serum levels of a soluble form of Fas have been reported in patients with heart failure, and high levels are associated with severe disease.[11].The administration of a nonspecific immunomodulating agent pentoxifylline[12] or intravenous immunoglobulin reduces plasma levels of Fas as well as C-reactive protein and is reported to improve left ventricular function in patients with ischemic or dilated cardiomyopathy.

Neurohormones

In the early 1960s it was reported that patients with heart failure had abnormally elevated levels of plasma norepinephrine at rest and that further elevations occurred during exercise. The urinary excretion of norepinephrine was also increased.[14] These findings suggested that the sympathetic nervous system is activated in patients with heart failure and that a neurohormonal disturbance might play a pathogenetic role in heart failure.

The Valsartan Heart Failure Trial (Val-HeFT) investigators compared the prognostic values of plasma neurohormones among 4300 patients.The most powerful predictors of

mortality and hospitalization for heart failure, after BNP, were big endothelin-1, followed by norepinephrine, endothelin-1, plasma renin activity, and aldosterone.[15]

In the Randomized Aldactone Evaluation Study (RALES) of patients with severe heart failure, it was found that administration of the aldosterone blocker spironolactone was associated with a reduction of plasma procollagen type III and clinical benefit[16]

Although elevated levels of several neurohormones can be used to predict adverse outcomes in patients with heart failure, they are relatively unstable in plasma and may be difficult to measure on a routine basis.[11]

Myocyte Injury

Myocyte injury results from severe ischemia, but it is also a consequence of stresses on the myocardium such as inflammation, oxidative stress, and neurohormonal activation. Modest elevations of cardiac troponin levels are also found in patients with heart failure without ischemia. Cardiac troponin I was detectable ($\geq$ 0.04 ng per milliliter) in approximately half of 240 patients with advanced, chronic heart failure without ischemia.The presence of cardiac troponin I remained an independent predictor of death[11]

Adrenomedullin

Adrenomedullin is a peptide of 52 amino acids and a component of a precursor, pre-proadrenomedullin, which is synthesized and present in the heart, adrenal medulla, lungs, and kidneys.It is a potent vasodilator, with inotropic and natriuretic properties, the production of which has been shown to be stimulated by both cardiac pressure and volume overload.[11]

ST2

ST2, a member of the interleukin-1 receptor family, is a protein secreted by cultured monocytes subjected to mechanical strain.Elevated levels of ST2 occur in patients with severe heart failure. In patients presenting to the emergency department with myocardial infarction with ST elevation and dyspnea, ST2 levels were strongly predictive of mortality.[11]

New Biomarkers

Biomarkers other than those already discussed are under investigation. These include

Chromogranin A- a polypeptide hormone produced by the myocardium(has potent negative inotropic properties)

Galectin-3 - protein produced by activated macrophages (predict adverse outcomes in patients with heart failure)

Osteoprotegerin- a member of the tumor necrosis factor receptor superfamily (implicated in the development of left ventricular dysfunction)

Adiponectin- a 244-amino-acid peptide(elevated in patients with advanced heart failure)

Growth differentiation factor 15-predicts the risk of death in patients with heart failure

ATHEROSCLEROTIC RISK FACTORS

High Sensitivity CRP

CRP was discovered in 1930 by Tillet and Francis.It is derivred primarily from the liver,but cells of the coronary intima,especially atherosclerotic intima can also elaborate CRP.CRP is a acute phase reactant and its values rise rapidly and reaches its peak within 24-48 hrs.Its half life is 19 hrs hence levels decrease rapidly on resolution of the inflammatory stimulus.[3]

CRP is not only an inflammatory marker of atherosclerosis but also actively participates in process of atherogenesis, and it was found in higher levels in those who later developed

cardiovascular events than those who did not.Hence it was shown that addition of hsCRP measurement to the Framingham Risk Score adds additional prognostic information.[21]

hsCRP levels are increased with Raised BMI, Cigarette smoking. Metabolic syndrome & DM, Low HDL & high triglycerides, Oestrogen /progesterone harmone use. The levels are decreased withModerate alcohol consumption, Increased activity, Weight loss.

AHA & CDC issued guidelines in 2003 for the use of hsCRP in clinical practice. hsCRP levels less than 1,1to 3,& more than 3 mg/litre should be interpreted as lower, moderate & higher vascular risk along with traditional markers of risk.[22].Values more than 8 mg/litre may represent an acute phase response caused by an underlying inflammatory disease or intercurrent infection, and repeat testing is needed in approximately 2-3 weeks. Consistent high values however represent very high risk for future cardiovascular events.

Levels of hsCRP greater than 3 mg/litre also predict recurrent coronary events, thrombotic complications after angioplasty, poor outcomes after CABGS. Hence individuals with elevated hsCRP levels are more likely to benefit from aggressive interventions compared to those with low hsCRP[23]

Statins reduce hsCRP levels in a manner unrelated to LDL reduction,and studies have shown that event reduction with statins may be greater in presence of elevated hsCRP levels.

In the AFCAPS/TexCAPS trial statins reduced event rates even for those with below median levels of LDL but above median level of hsCRP.By contrast statins did not reduce event rates in those with neither hyperlipidemia or inflammation.[24].In the PROVEIT-TIMI 22 clinical trial in patients with ACS achieving levels of hsCRP less than 2 mg/lit was as important as achieving LDL less than 70 mg/dl.Infact the best outcomes was seen in patients who achieved the dual goal.[25].In the A to Z trial where intravascular ultrasound was used to monitor atherosclerosis regression,only those with CRP reduction with statin had coronary regression & those who achieved both CRP & LDL reduction had greatest regression.[26]

It is proposed that hsCRP estimation should be routinely done at the time of cholesterol estimation and individuals with elevated hsCRP and LDL greater than 160 mg/dl should treated aggressively treated with pharmacotherapy and life style modifications.To achieve a hsCRP level of less than 2 mg/dl is as important as reaching a LDL level less than 70 mg%.

Homocysteine

Homocysteine is a sulphydryl containing amino acid derived from demethylation of dietary methionine.

Mild to moderate elevations of homocysteine levels (more than 15 umol/lit) are commonly seen with- Dietary deficiency of folic acid, Folate antagonist such as methotrexate, carbamazepine, Hypothyrodism, Renal insufficiency

Rare inherited deficiency of methionine metabolism can develop severe hyperhomocysteinemia (more than 100 umol/lit) and have markedly elevated risk of premature atherothrombosis, and venous thromboembolism. Mechanisms suggested are Endothelial dysfunction, Accelerated oxidation of LDL, Impairment of flow mediated endothelium derived relaxing factor, Platelet activation, Oxidative stress.

Though increased levels of homocysteine are associated with increased vascular risk, yet clinical trials of homocysteine reduction have not shown substantive benefit, in both patients with stroke and coronary artery

disease.[27,28,29]

Despite lack of evidence that there remains a few patient specific population in whom homocysteine decrease may be beneficial; like in those lacking traditional risk factors with renal failure; patients with premature atherosclerosis, or family history of MI or stroke at a young age.

LIPOPROTEIN (a)

Lipoprotein (a), Lp(a) consists of an LDL particle with its apolipoprotein B-100 [apo B-100] component linked by a disulphide bridge to apolipoprotein (a), apo (a), a variable length protein that has sequence homology to plasminogen. The close homology between Lp(a) and plasminogen has raised the possibility that this lipoprotein may inhibit endogenous fibrinolysis by competing with plasminogen binding on the endothelium.

Increased levels of Lp(a) are associated with an increased risk of CHD ,and it was found to be an independent predictor of stroke, death from vascular disease & death from any cause in men.[37,38]

But Lp(a) is not routinely screened because with the exception of high dose niacin few interventions lower Lp(a) and no study shows that Lp(a) reduction lowers vascular risk.

URIC ACID

Uric acid is the major product of purine metabolism and is formed from xanthine. Studies have shown elevated uric acid levels predict an increased of cardiovascular events.

In a prospective cohort study, in which the mean follow-up was 1.9 years, it was found that serum uric acid level are a strong predictor of cardiovascular mortality in healthy middle aged men. But whether the relationship to cardiovascular events was circumstantial or causal remains to be answered.[3]

SERUM AMYLOID A

Serum amyloid A (SAA) is a family of proteins that form a major component of the acute-phase inflammatory response. Like C-reactive protein (CRP), SAA is synthesized in the liver in response to infection, inflammation, injury, or stress. SAA like CRP has been linked to atherosclerosis.

In the Women's ischaemia Syndrome Evaluation (WISE) study a strong independent relationship between SAA and future cardiovascular events, similar to that for hs-CRP was found. SAA was independently but moderately associated with angiographic CAD.[32]

Extracellular-Matrix Remodeling

Remodeling of the ventricles plays an important role in the progression of heart failure.[30] The extracellular matrix provides a "skeleton" for myocytes and determines their size and shape[13]

At least 15 matrix metalloproteinases and several forms of procollagen and of tissue inhibitors of metalloproteinases have been identified. Which of these are the most informative and appropriate for routine measurement requires clarification.

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