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Novel biomarkers for risk stratification of acute coronary syndromes

Ram S Kaulgud*, Vijayalxmi P B, Arun B S, Supriya Rao R and Vigneshwar M

Department of Internal Medicine, Karnataka Institute of Medical Sciences, Hubballi, India

***Correspondence Info:**
Dr. Ram S Kaulgud,
Assistant Professor,
Department of Internal Medicine,
Karnataka Institute of Medical Sciences, Hubballi, India
E-mail: ramk72@yahoo.com

Abstract

Acute coronary syndromes are one of the most important causes of death world-wide. The clinical diagnosis, though is correct in majority of the cases, it is not cent percent, which is not acceptable when the life of the patient is at stake. Various biomarkers have been tested and found useful in diagnosing, ruling out, prognostication and risk stratification of the acute coronary syndromes. These biomarkers have the potential to change the management of the acute coronary syndromes, making the management of acute coronary syndromes more precise and more accurate.

Keywords: biomarkers: prognosis: risk stratification.

1. Introduction

Atherosclerosis is a process of narrowing of the lumen of the coronary arteries due to deposition of lipids. Coronary heart disease is clinical manifestation of this pathophysiologic process. Acute coronary syndromes are the most common cause of mortality world-wide. These comprise of stable angina, unstable angina, myocardial infarction. These are usually diagnosed based on certain clinical features, supported by biomarkers like Troponins. Though the currently available blood tests (Creatine Phosphokinase and cardiac troponins) differentiate angina from myocardial infarction, differentiating non cardiac chest pain from stable and unstable angina objectively and definitely is not yet possible. There are certain grey areas, for diagnosis of which current management guidelines prove inadequate. Several biomarkers are being tried to get the answers for these difficult but life/death questions. But, until now, no single biomarker has been proven to predict adverse cardiovascular events in patients with acute coronary syndrome. So, the emphasis has now shifted toward "multi-biomarker" approach, which consists of using a panel of biomarkers to accurately identify cardiac chest pain and to predict the prognosis of a patient for cardiovascular disease as well. Several inflammatory molecules are being tested as candidate biomarkers for plaque vulnerability, which is a key pathophysiologic feature of acute coronary syndromes. Some biomarkers are indicators of early myocardial ischemia and some are indicative of myocardial dysfunction and some are predictors of short/long term prognosis.

2. Classification of important cardiac biomarkers

I. Markers of inflammation
- i. Myeloperoxidase
- ii. C Reactive Protein
- iii. Soluble CD40 Ligand
- iv. Matrix metalloproteinases

II. Plaque vulnerability
- i. Pregnancy Associated Plasma protein A
- ii. Placental growth factor

III. Myocardial damage
- i. Troponins I and T
- ii. Creatine phosphokinase
- iii. Fatty Acid Binding Protein

IV. Myocardial Ischemia- Ischemia Modified Albumin

V. Myocardial dysfunction
- i. Brain natriuretic peptide
- ii. N terminal Brain natriuretic peptide

2.1 Copeptin

Copeptin, a 39-amino acid glycopeptide that comprises the C-terminal part of the AVP precursor

(CT-proAVP), was found to be a stable and sensitive surrogate marker for AVP release, analogous to C-peptide for insulin.[1]

2.1.1 Biological role

Once secreted into the bloodstream, there is no known biological role for copeptin. However, when pre-pro-vasopressin is processed during the axonal transport, copeptin may contribute to the 3D folding of vasopressin[2].

The clinical interest in copeptin testing is closely linked to the pathophysiological pathways in which vasopressin is involved : polydipsia-polyuria syndrome, hyponatremia, SIADH as well as heart failure and acute coronary syndrome.[3]

It has a longer half-life, thus potentially better marker of the upregulation of the arginine-vasopressin system in cardiac ischemia

2.1.2 Clinical use

Copeptin ≤14pmol/L in combination with cTnT≤0.01µg/L, has the sensitivity of 98.8% and negative predictive value of 99.7% in patients who presented with acute MI<12h of onset [4].

Copeptin may have not only short term prognostic relevance, but also identify patients at higher long-term risk. Increased copeptin concentrations in elderly patients with symptoms of heart failure were associated with an increased risk of all-cause mortality after a lengthy median follow-up of 13 years [5].

2.2 CRP

CRP is a non specific inflammatory marker released as an acute phase reactant secondary to necrosis. It is a member of pentraxin family of proteins and is synthesized in the liver in response to IL-6.[6][7] Discovered by Tillett and Francis in 1930[8]. It is a 224 residue protein so named due to its capacity to precipitate somatic C polysaccharide of Streptococcus Pneumoniae.[9] Specimen- whole blood, serum, plasma. Collection- Red top/ serum separate tube. Levels of CRP are increased in-acute and chronic infections, tissue necrosis or injury, ischaemia/infarcts, metabolic syndrome, malignant tumours of breast, lung and git, acute pancreatitis, post surgery, burns, leukaemia, obesity, HRT. CRP levels provide additional value in prognosis of ACS however the incremental value is modest. Reference range- CRP- 0 to 10 mg/dl and hsCRP- - <3 mg/dl. Hs CRP starts increasing within one or two days of AMI, peaks at 3 days and becomes negative after 7 days. Failure to return to normal signifies tissue damage in cardiac and other tissues. Absence of CRP raises the question of necrosis prior to 2 to 10 days. In AMI, CRP levels correlate with infarct size, mortality and cardiac complications. Levels of CRP do not usually increase in unstable angina.

High levels of CRP can be a marker of atherosclerosis. Hs CRP is an important predictor of CV events including MI, CVA, PVD and sudden cardiac death in an individual without a history of heart disease. High CRP carries a worse prognosis in a patient with ACS and the mortality related to these levels is independent of LV function. Limitation- Sex and race can affect CRP levels: African Americans have higher values compared to Caucasians and women more than men. Consideration- For its utility as a CV risk marker, the values need to be measured 2 times at least 2 weeks apart in a metabolically stable state, post infection or illness as its half life is 19 days. The best evidence to date supports the use of hs-CRP as an independent predictor of high risk for coronary artery disease.[10] The cut points of low risk<1.0mg/L>, average risk<1 to 3mg/L> and high risk< >3 mg/L correspond to approximate tertiles of hs-CRP in the adult population.[6]

2.3 Heart -type fatty acid binding protein

Heart-type fatty acid-binding protein (H-FABP) is a small membrane-bound cytoplasmic protein that facilitates transport of fatty acids from the blood into the heart. Because of its small size, H-FABP is released quickly into the circulation when membrane integrity is compromised in response to cardiac ischemia. Levels of H-FABP are detectable as early as 2 to 3 hours after injury, with a return to baseline levels typically within 12 to 24 hours of the initial insult.[11] Although both myoglobin and H-FABP are present in cardiomyocytes, a much higher proportion of H-FABP is concentrated in myocardial tissue cells relative to myoglobin.[12] It is currently being used outside the United States for the early diagnosis of myocardial infarction (MI).[13] Specifically and reversibly bind to long chain fatty acids. Its content in skeletal muscles is only 10-30% of that of cardiac muscles. The diagnostic potential of the biomarker H-FABP for heart injury was discovered in 1988 by Professor Jan Glatz (Maastricht, Netherlands)[3] H-FABP is 20 times more specific to cardiac muscle than myoglobin[14]

2.3.1 Clinical use

Greater value and sensitivity than cTnT, CKMB, Myoglobin in patients with acute coronary syndrome. H-FABP is recommended to be measured with troponin to identify myocardial infarction and acute coronary syndrome in patients presenting with chest pain. H-FABP measured with troponin shows increased sensitivity of 20.6% over troponin at 3-6 hours following chest pain onset.[15] H-FABP has been proven to significantly predict 30 day mortality in acute pulmonary embolism[16] H-FABP is more

effective than Troponin T in risk stratifying Chronic Heart Failure patients[17].

2.4 Ischaemia modified albumin (IMA)

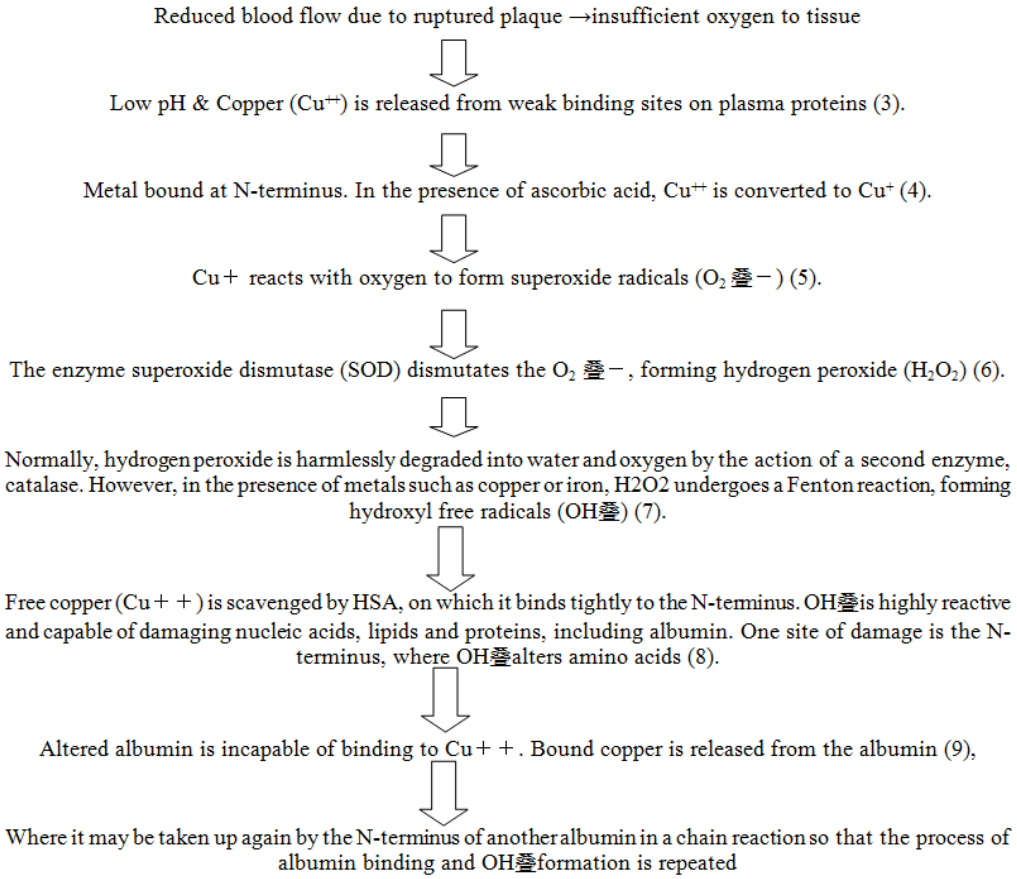
Under physiological conditions, transition metals can bind tightly to the exposed N-terminus of albumin.[18] In the presence of myocardial ischemia, structural changes take place in the N-terminus of the protein,which reduce its binding capacity, possibly, in part, as a result of exposure to reactive oxygen species (ROS). Ischaemia induces a conformational change in albumin, so that it can no longer bind to transitional metals such as cobalt or copper.

Using the albumin cobalt binding (ACB) test, the quantum of ischaemia modified albumin can be estimated and this serves as an index of ischaemia.

Ischaemia-modified albumin (IMA) has been shown to be an independent predictor of short- and long-term adverse outcomes over and above conventional known risk in patients with ACS. Increased IMA values may be found in patients with cancer, infections, end-stage renal disease, liver disease, and brain ischaemia also.

The commercially available IMA test appears to be relatively sensitive for identifying unstable angina. However, the test's specificity is relatively poor and the assay is cumbersome to use. With greater refinement it may be a useful test in the emergency department (ED) to rule out ischemia which is more important at that stage.[19]

Figure 1: Postulated mechanism of IMA generation [20]



2.4.1 Clinical use

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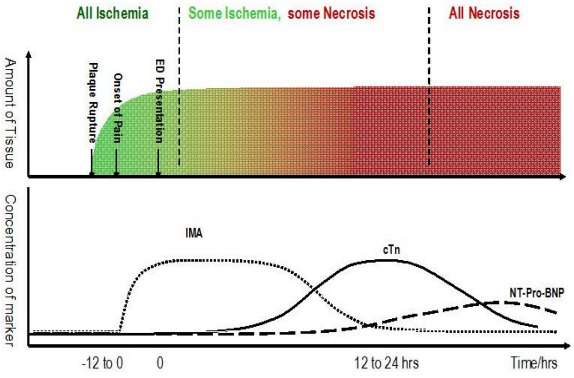
2.4.2 Release kinetics of IMA in relation to standard cardiac biomarkers of necrosis (cardiac troponin, cTn) and dysfunction (B type natriuretic peptide, BNP):

Rises within minutes of ischemia, stays up for 6-12 hrs and normalises within 24hrs. The rapid reduction in IMA following the ischemic event suggests the modification is transient and reversible.[20] IMA alone has a diagnostic sensitivity of 82% with 46% specificity if measured in the first three hours of presentation to the ED. The

combination of a positive ECG, cTnT (>0.05 µg/L) and IMA however demonstrated a 95% sensitivity for diagnosis of ACS.[21]

Figure 2: Release kinetics of IMA in relation to standard cardiac biomarkers of necrosis



2.5 CD40 Ligand

CD40L (L stands for ligand), a trimetric transmembrane protein of the tumor necrosis family also known as CD154. CD40L is also stored in platelets and translocated to the platelet surface upon stimulation within seconds after activation in vitro and *in vivo*. sCD40L can promote inflammatory or thrombotic response by causing further platelet activation. This can occur through binding to platelet CD40 or to the integrin. More than 95% of the circulating CD40L exists in platelets, and the role of platelets was already explained as inflammatory cells.

Higher levels of sCD40L, IL-6, and thrombotic markers exist in MetS patients, particularly those with IHD. The strong positive correlations between sCD40L and IL-6, TF, and platelets count support a link between the CD40–CD40L system and the underlying inflammatory and hypercoagulable state in MetS patients.[22]

Elevation of CD40L identifies the patients who are at highest risk for cardiac events and who are likely to benefit from treatment with the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonists. Several studies demonstrated that the circulating level of CD40L was related to a high thrombus burden in acute MI and long-term mortality. Cigarette smokers also have upregulation of the CD40/ CD40L dyad. However, there is no report about circulating levels of CD40L in smokers with acute MI. Circulating levels of sCD40L are increased in smokers during the early phase of acute MI. Therefore, smokers with acute MI may have an increased risk of thrombotic complications during acute MI.[23]

2.6 Myeloperoxidase

Myeloperoxidase (MPO) has emerged as an important pathophysiological determinant of inflammatory vascular artery disease. It is appreciated that vessel immobilized, rather than circulating, MPO is critical for the progression of atherosclerotic lesions. Administration of heparin, which is strongly anionic, releases MPO from the vessel wall. Vascular MPO deposition is closely linked to the overall coronary plaque burden in patients with stable CAD, thus strengthening the pathophysiological role of MPO in coronary artery disease [24] Myeloperoxidase and protein carbonyl levels are elevated in plasma after acute MI, apparently via independent mechanisms. High MPO is a risk factor for long-term mortality and adds prognostic value to LVEF and plasma NT-proBNP measurements. The MPO levels were elevated in plasma obtained from patients with MI, 24 to 96 h after admission compared with control plasma.

High levels of MPO were independently prognostic of mortality over a 5 year follow-up and, in combination with low LVEF or high plasma NT-proBNP, were associated with an even greater mortality than any of these risk factors alone. Myeloperoxidase shows promise as a prognostic marker of long-term mortality in patients with a confirmed MI diagnosis, particularly when used in combination with the other established markers.[25]

2.7 Matrix Metalloproteinase-9

Experimental evidence suggests that matrix metalloproteinase-9 (MMP-9) may play a causal role in new-onset cardiovascular disease (CVD). MMP-9 may contribute to weakening and rupture of atherosclerotic plaques. MMP-9 (also known as gelatinase B, 92 kDa collagenase) is one of a family of endopeptidase enzymes involved in the degradation and re-organisation of the extra-cellular matrix which is involved in vascular remodeling; inappropriate or remodeling can promote atherosclerosis or restenosis. MMPs are expressed in cells including macrophages, endothelial cells and vascular smooth muscle cells and have been identified in the shoulder area of human plaques where MMPs may promote the fibrous cap to weaken, destabilizing the plaque. MMP-9 was associated with modestly elevated risks of MI and stroke, in large part due to confounding, particularly by cigarette smoking and inflammation.[26] There is a significant association between serum MMP-9 and incident CHD in the general population. This is largely related to cigarette smoking exposure and to circulating markers of generalized inflammation. The association is of biological interest and is consistent with a potential role for MMP-9 in pathogenesis of CHD. However, MMP-9is unlikely to be a clinically useful additional risk predictor for CHD in the general population.

2.8 Pregnancy- associated plasma Protein-A

It is also known as Pappalysin -1[27] Zinc dependent matrix metalloproteinase[28] abundantly expressed in ruptured plaque but minimally expressed in stable plaque. PAPP-A levels are significantly higher in patients with unstable angina or acute MI than in patients with stable angina or controls.[29] Association between PAPP-A levels & free IGF-1 levels the recent identification of PAPP-A as the enzyme cleaning IGF- binding protein-A, an inhibitor of the action of IGF, suggests that PAPP-A increases the availability of IGF-I, thus contributing to the progression of bulk coronary atherosclerosis & restenosis. The free fraction circulating & locally synthesized IGF-I induced the migration of vascular smooth muscle cells & is important of monocyte chemotaxis& activation & release of cytokines within atherosclerotic lesion. [30]

2.9 Placental Growth Factor

Member of vascular endothelial growth factor family that is strongly upregulated in atherosclerotic lesions & acts as a primary inflammatory investigator of atherosclerotic plaque instability. In patients with ACS, increased plasma levels of PIGF are associated with adverse cardiac outcomes during loy teens follow up.[31]

2.10 NTpro BNP

The Nterminal of the prohormone brain natriuretic peptide (NTproBNP), also commonly abbreviated BNPT, is a 76 amino acid N terminal inactive protein that is cleaved from proBNP to release brain natriuretic peptide.

2.10.1 Biological role

BNP represents the active hormone and when it is released from myocytes acts to reduce hemodynamic stressors such as wall stretch through natriuresis, vasodilation, inhibition of the rennin-angiotensin-aldosterone axis and sympathetic nervous system.[32] NTproBNP, on the other hand, is an inactive cometabolite of the common intracellular precursor.[33] A metaanalysis of 12 studies that included patients presenting to the hospital with NSTE ACS showed that risk of death was 4.89fold greater in those patients with elevated NTproBNP levels on admission.[34] NTproBNP's use in combination with cTn has been shown in studies to improve the diagnostic ability of clinicians to differentiate between MI, unstable angina, and noncardiac causes of chest pain.[35] In low risk patients, combining cTn and NTproBNP (or perhaps BNP) in a "ruleout" biomarker based model may provide the opportunity to safely discharge these patients without the current standard of care, stress test, saving the individual patient and healthcare system much aggravation and cost.[36] NTproBNP are raised in both symptomatic and asymptomatic patients with left ventricular dysfunction.[37] Recent smaller studies suggest that BNP and NTproBNP may be superior to ANP and NTproANP in the detection of early left ventricular dysfunction. [38] Measurement of NT pro BNP is advantageous compared to BNP as it is more stable after collection and upon long term freezing; NTproBNP also has a longer biological half life.[39] NTproBNP provides information that may be superior to clinical judgment for the diagnostic evaluation of the patient with possible HF. It is a surrogate biomarker for prognosis after STEMI that is closely associated with myocardial damage as assessed by contrast enhanced Cardiac MRI.[40] NTproBNP's ability to indicate structural heart disease along with its correlation with ACS, infarct severity and prognostic implications post MI confer the potential for its use in a variety of roles in the evaluation and management of this disease process.[41] BNP and NTproBNP are most commonly associated with clinical role in the diagnosis or rule out of congestive heart failure, they have been evaluated for use in MI for prognostication, risk stratification, and rule out of ACS in low risk patient.[42]

3. Conclusion

There is enough scientific evidence available for recommending regular use of the above mentioned biomarkers for management of acute coronary syndromes. There is need for larger studies in our country to study the cost-effectiveness of these biomarkers in management of acute coronary syndromes in Indian context.

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