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New Biochemical Markers of Ischemia: The Diagnostic and Prognostic Role of Brain Natriuretic Peptide, C-Reactive Protein, Ischemia Modified Albumin & Myeloperoxidase

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ABSTRACT

The traditional biomarkers, CK-MB and troponins, used for the diagnosis of myocardial ischemia and the risk stratification of patients with acute coronary syndromes (ACS) are of limited use mainly because they require some degree of necrosis in order to become detectable. Based on the knowledge gained into the pathophysiology of ACS, several new biomarkers have been developed. Brain natriuretic peptides (BNP and NT-proBNP) as markers of hemodynamic stress have shown in several studies a good diagnostic and prognostic performance. C-reactive protein (CRP) reflecting systemic inflammation, has mainly a role as risk stratifier. Ischemia modified albumin (IMA) a pure ischemia marker may offer a substantial aid in diagnosing an acute coronary event in patients with negative troponin. Finally, myeloperoxidase (MPO), a marker of oxidative stress, may also contribute to the diagnosis of ischemia although this is not yet supported by a fair amount of data. Nevertheless, although highly sensitive, these new biomarkers are not specific enough and a multi-marker approach seems the most appropriate strategy for diagnosis of myocardial ischemia and for assessing the risk of an adverse outcome in patients with an acute coronary syndrome.

List of Abbreviations:
ACS: acute coronary syndrome
ECG: electrocardiogram
CK-MB: creatine kinase-MB
Tn: Troponin
BNP: Brain Natriuretic Peptide
CRP: C-Reactive Protein
IMA: Ischemia Modified Albumin
MI: myocardial infarction
MPO: Myeloperoxidase
NT-proBNP: N-terminal fragment – proBrain Natriuretic Peptide
MI: Myocardial Infarction
NYHA: New York Heart Association
LDL: Low Density Lipoprotein
ROC: Receiver Operator Characteristic

KEY WORDS: *acute coronary syndromes; inflammation; risk stratification; chest pain; biomarkers*

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INTRODUCTION

Establishing a diagnosis of myocardial ischemia in the clinical setting remains a challenging task. In addition to history of chest pain and abnormal electrocardiographic (ECG) changes, laboratory evidence of myonecrosis has always been an integral part of the initial diagnostic work up of a suspected acute coronary syndrome (ACS) [1]. Unfortunately, the myonecrosis markers, myoglobin, creatine kinase (CK-MB) and the troponins (Tns), cannot by definition help clinicians in the assessment of patients with stable or unstable angina where ischemia is not accompanied most of the time by myocardial necrosis. In addition, myocardial necrosis is time-dependent, such that these highly sensitive and specific markers might give negative results on admission but give positive results hours later [2]. As such, the usefulness of the conventional biomarkers of myocardial necrosis for the confident exclusion of the diagnosis of myocardial ischemia at the time of admission remains limited. Markers able to identify

patients with myocardial ischemia without infarction might play an important role in the clinical setting since among those patients with a definite ACS, early treatment may reduce the extent of myocardial injury, and thus rapid diagnosis and initiation of therapy is a central tenet of management [3]. In addition, given the increasing array of treatments for the heterogeneous population of patients admitted with ACS, effective risk stratification and targeting of therapy have become a focus of contemporary management of clinically evident myocardial ischemia [4]. As such, the objectives of the initial assessment are twofold: (a) to assess the probability that the patient's symptoms are related to acute coronary ischemia (i.e. establish the diagnosis) and (b) to assess the patient's risk of recurrent cardiac events, including death and recurrent ischemia (define the prognosis). In this review, the role of some newly developed biochemical markers in establishing the diagnosis and prognosis of myocardial ischemia will be briefly presented.

BRAIN NATRIURETIC PEPTIDES (BNP)

The brain natriuretic peptide (BNP) is synthesized by cardiac myocytes when left ventricular wall stress increases [5]. After secretion, the pro-hormone is cleaved to the biologically active hormone (BNP) and to the inactive N-terminal fragment (NT-proBNP). Enough evidence already exists that measuring the blood level of either one of these molecules improves the ability to diagnose or exclude heart failure as the cause of acute dyspnea [6,7]. Moreover, BNP measurement provides useful prognostic information on mortality risk in patients with heart failure [8,9]. However, only recently BNP has also been recognized as a potential diagnostic marker of myocardial ischemia as well as a prognostic indicator in patients with coronary artery disease. Jernberg et al [10] collected blood samples of 775 acute chest pain patients without ST-segment elevation upon admission to the coronary care unit and showed that patients with an acute myocardial infarction (MI) had significantly higher median BNP levels than patients with unstable angina or non-cardiac chest pain. Bassan et al [11] studied prospectively 631 patients presenting to the emergency department with chest pain and without ST-elevation in the ECG. Sensitivity of admission BNP for acute MI (cut off value of 100 pg/ml) was significantly higher than CPK-MB and TnI (70.8% vs. 45.8% vs. 50.7% respectively). However, specificity was substantially lower (~70% vs. ~98%). Simultaneous use of all 3 markers significantly improved sensitivity to 87.3% and negative predictive value to 97.3%. According to these data, the use of natriuretic peptides in the emergency department as a simple aid for deciding whether a chestpain patient should be admitted to the hospital or can safely be discharged home seems very promising. Nevertheless, this marker needs to be more thoroughly investigated before being accepted as a clinically useful tool.

On the other hand, more research has been conducted regarding the prognostic role of natriuretic peptides in the setting of acute or chronic myocardial ischemia. Levels of BNP and NT-proBNP have shown to correlate with left ventricular dilatation, remodeling, and dysfunction, as well as congestive heart failure and death among patients presenting with acute MI [12]. Beyond that, several studies have now demonstrated a robust association between BNP or NT-proBNP and the short- and long-term risk of death across the spectrum of non-ST-elevation ACS including patients without myocardial necrosis or clinical evidence of heart failure. In the most recent of those studies, Jarai et al [13] showed that Nt-proBNP along with TnI were independent predictors of 2-year mortality in 120 patients with unstable angina. These findings confirmed the results of previous studies which had similarly shown that both BNP and NT- proBNP offered significant information about short and long term prognosis in patients with unstable angina and that this information was independent of the left ventricular systolic function [14-17].

Finally, another very interesting emerging concept is that natriuretic peptides may have a prognostic role not only in the acute ischemia setting but also in patients with chronic ischemic heart disease. Thus, Ndrepepa et al [18] recently published the results of their study which included 1059 patients with chronic stable angina. NT-proBNP levels were the strongest correlate of 5-year mortality in this population outweighing other significant variables like age, New York Heart Association (NYHA) class and CRP. In this study, plasma NT-proBNP enabled the identification of a group of patients who were at particularly high risk for death after coronary intervention with stenting. Similar findings were reported by the AtheroGene study investigators who prospectively followed 904 patients with stable and unstable angina for 2 years [19]. Baseline NT-proBNP was significantly higher among individuals with cardiovascular events compared with those without. In the subgroup of patients with stable angina, those within the top quartile had a 3.7-fold increase in cardiovascular risk. To further expand the prognostic role of natriuretic peptides even in patients with disease in other than the coronaries vascular beds, Campbell et al [20] showed that NT-proBNP levels were able to predict MI in subjects who had experienced a cerebrovascular event. In this nested case-control study which was part of the Perindropril Protection Against Recurrent Stroke Study (PROGRESS), NT-proBNP was found superior to CRP and to renin levels in predicting cardiovascular risk. The mechanism responsible for the increased levels of brain natriuretic peptides in myocardial ischemia remains unclear. Increased ventricular wall stress due to ischemia induced systolic or diastolic dysfunction certainly plays a role. Indeed, Richards et al [21] recently showed that both BNP and NT-proBNP closely correlated with left ventricular ejection fraction in a large population with stable

ischemic heart disease being at the same time independent predictors of 12-month total mortality and admission to the hospital with heart failure. However, evidence also exists that ischemia may itself promote BNP gene expression in the hypoxic myocardium of the left ventricle [22].

C-REACTIVE PROTEIN

C-reactive protein (CRP) is the inflammatory marker receiving the most attention to date as a prognostic indicator of coronary artery disease. It is an acute phase reactant normally present in plasma at low levels, and increases >100-fold in response to inflammatory stimuli. It is produced by hepatocytes in response to stimulation by interleukin-6. It is also produced by human coronary artery smooth muscle cells [23]. Although initially considered only a marker of inflammation, CRP itself has been shown to possess pro-inflammatory and pro-atherogenic properties. It stimulates endothelial cells to express adhesion molecules and secrete cytokines [24,25] and it decreases the expression of endothelial NO synthase [26,27]. CRP accumulates in macrophage-rich regions of nascent atherosclerotic lesions and activates the macrophages to express cytokines and tissue factor, while enhancing macrophage uptake of LDL [28]. It also amplifies pro-inflammatory effects of several other mediators including endotoxin [29,30]. In a post-mortem study of 302 autopsies of men and women with atherosclerosis, median CRP levels were higher with acute plaque rupture than in stable plaques or controls [31]. The levels correlated with the staining intensity for CRP in macrophages and the lipid core of plaques, and it increased with the number of thin cap atheromas found in coronary arteries. Plasma CRP levels at the upper end of the reference range in apparently healthy men and women, in the absence of other sources of inflammation, correlated with increased risk of future cardiovascular events, including MI, peripheral vascular disease with intermittent claudication and stroke [32]. These data support the view that systemic CRP accurately reflects the number of vulnerable atherosclerotic plaques. A decade ago Liuzzo et al [33] reported that patients with unstable angina and elevated levels of CRP (>3 mg/dl) had higher rates of death, acute MI and need for revascularization compared to patients without elevated levels. Moreover, this increased risk may be evident as early as 14 days after presentation [34]. These findings have been confirmed by more recent data. The CAPTURE trial found that although only TnT was predictive in the first 72-hour period, both CRP and TnT were predictors of risk within 6 months [35]. The FRISC Investigators reported that the risk associated with elevated CRP levels at the time of an index ACS event continued to increase for several years afterwards [36]. Mueller et al [37] reported that in ACS patients who were treated with very early revascularization, CRP was a strong independent predictor of both short-term and long-term mortality. However, not everybody agrees that CRP levels are of significant prognostic importance. Lee et al [38] recently showed that CRP did fairly worse compared to interleukin-6 and total homocystein in predicting coronary artery disease related death in a cohort of 1117 consecutive patients undergoing selective coronary angiography. Nevertheless, most of the experts in the field seem to agree that the role of CRP as a prognostic marker in patients with an ACS appears to be established.

Unfortunately, despite its prognostic importance, the contribution of CRP to the diagnosis of coronary ischemia is rather poor. This has been confirmed by a recently published systemic review of the potential use of 22 protein markers in low risk patients presented to the emergency department with chest pain. CRP demonstrated an area under the curve of only 0.61 in the summary Receiver Operator Characteristic (ROC) curve analysis with a pooled diagnostic odds ratio of 1.81 [39]. This has to be attributed to the very low specificity of this biomarker. Daily fluctuations in basal CRP levels are significant and are 4–6 times greater than cholesterol fluctuations. CRP levels are transiently elevated for 2–3 weeks following a major infection or trauma. Chronic inflammatory conditions like rheumatoid arthritis or lupus will also confuse interpretation of CRP levels. Minor inflammatory stimuli, such as viral infection, skin lacerations and some noninflammatory states (e.g., a low level of physical activity, aging, chronic fatigue, high protein diets, alcohol consumption and depression), are also known to influence CRP. These limitations make the CRP practically useless in differentiating among patients with chest pain those who have myocardial ischemia. Moreover, knowledge of these other causes of CRP fluctuations can help interpret its value for cardiovascular risk assessment as well.

ISCHEMIA MODIFIED ALBUMIN (IMA)

Ischemia modified albumin (IMA) is a new marker of transient myocardial ischemia. IMA is measured by the Albumin Cobalt Binding (ACB) test, which measures the binding capacity of exogenous cobalt to the N-terminus of human albumin. In the presence of myocardial ischemia, structural changes take place in the N-terminus of albumin that rapidly reduce its binding capacity for transition metal ions. These changes in the N-terminus of human albumin are attributed, among other factors, to ischemia/reperfusion mediators, hypoxia, and acidosis. There is no correlation between the ACB test results and human serum albumin levels in normal range. Studies have shown that IMA is highly sensitive for the identification of ACS and, in combination with the ECG and troponin, has both high sensitivity and negative predictive value. IMA has also been shown to be elevated in patients after coronary angioplasty as a result of ischemia reperfusion injury.

Unlike troponin, a marker of ongoing myocardial injury,

IMA is a marker of impending myocyte necrosis. During ischemia, free-radical damage alters the ability of albumin to bind cobalt. Using a colour indicator (dithiotreitol) to detect added cobalt, the level of such altered albumin in serum can be quantitated [40]. IMA has been shown to rise within minutes after the onset of ischemia, stay elevated for 6 to 12 hours, and return to normal within 24 hours. Furthermore, IMA has been shown to predict with high sensitivity subsequent elevation in the Tns in the clinical setting [41]. Blood levels of IMA rise in patients who develop ischemia during percutaneous coronary intervention [42,43]. IMA levels during balloon angioplasty are related to number, pressure, and duration of inflations, suggesting that IMA reflects the magnitude and duration of ischemia induced during percutaneous coronary intervention and is not simply a marker of free radical damage.

Moreover, according to recent studies, IMA has twice the sensitivity of an ECG and four times the sensitivity of troponin to detect patients with ACS at time of presentation; this is particularly evident in those patients with unstable angina, which is difficult to diagnose with other diagnostic methods. Sinha et al [44] evaluated IMA in conjunction with ECG changes and cardiac TnT levels in 208 patients presenting to the emergency department within 3 hours of the onset of acute chest pain. In the whole patient group, sensitivity of IMA at presentation for an ischemic origin of chest pain was 82%, compared with 45% of ECG and 20% of TnT. IMA used together with troponin T or ECG, had a sensitivity of 90% and 92%, respectively. Similarly, Roy et al [45] showed that in 131 patients presenting to the emergency department with symptoms suggestive of acute myocardial ischemia but with normal or non-diagnostic ECGs, IMA levels >93.5 U/ml demonstrated a sensitivity and specificity of 75% for the diagnosis of ACS with an area under the ROC curve 0.78. Moreover, in combination with cardiac TnT levels >0.05 ng/ml, the sensitivity increased to 92.2%. These findings were confirmed by the recently published results of Anwaruddin et al [46] who measured IMA along with standard biomarkers (myoglobin, CK-MB and TnI) in 200 patients with suspected myocardial ischemia admitted to the emergency department. In this patient population, the myoglobin–CK-MB–TnI triad had a sensitivity of 57% for detecting myocardial ischemia. The combination of IMA–myoglobin–CK-MB–TnI increased the sensitivity for detecting ischemia to 97%, with a negative predictive value of 92%. It should be noted however, that IMA alone was somewhat poorly specific for the presence of ischemia (specificity: 31%). Given its high negative predictive value as supported by the current evidence, the test has been approved by the FDA as a "rule-out" marker of myocardial ischemia. But some questions about how timing affects its performance also persist [47]. The change to albumin binding occurs very quickly but it also seems to disappear quickly (within 2-3 hours of the ischemic event). The exact mechanism of the initial alteration in cobalt binding and the reason for its rapid disappearance is unknown. This limits IMA's usefulness as an additional marker in patients whose Tn level is only slightly elevated using the 99th percentile cut-off (and, therefore, possibly a false positive). If myocardial ischemia was the cause of the slight Tn elevation, IMA probably would have normalized by the time that Tn was elevated. Also, IMA may be falsely elevated (as far as myocardial ischemia is concerned) due to ischemia in other parts of the body [48].

Finally, the use of IMA as a risk stratifier in patients with acute or chronic ischemia has not been extensively evaluated. In the only study published so far, IMA was a poor predictor of serious cardiac outcomes in short term (72 hours of follow-up) [49]. Nevertheless, this was a rather small study of 189 patients and for a definite answer about the prognostic role of this new biomarker, further evaluation in larger trials seems necessary.

MYELOPEROXIDASE (MPO)

First identified within human atherosclerotic plaque nearly a decade ago [50], myeloperoxidase (MPO) has emerged as an important potential participant in the atherosclerotic process. MPO, a member of the heme peroxidase superfamily, generates reactive oxidants and diffusible radical species as part of its normal function in innate host defenses [51] A unique activity of MPO is its ability to use the halide chloride as co-substrate with hydrogen peroxide to generate chlorinating oxidants such as hypochlorous acid (HOCl), a potent antimicrobicidal agent [52]. MPO, and specific chlorinated protein and lipid oxidation products, are all markedly enriched within human atheroma. Leukocytes use MPO to generate oxidants capable of initiating lipid peroxidation [53] including conversion of LDL into an atherogenic form recognized by macrophage scavenger receptors [54]. MPO may also contribute to the atherosclerotic process by promoting endothelial dysfunction, by virtue of its capacity to catalytically consume nitric oxide as a substrate in vitro [55] and in vivo [56], resulting in formation of nitric oxide–derived oxidants [57].

Indeed, recent clinical studies demonstrate that systemic levels of MPO serve as a strong and independent predictor of endothelial dysfunction in subjects [58], as well as angiographic evidence of coronary artery disease [59]. Finally, recent human genetic studies support a potential role for MPO in coronary artery disease because MPO deficiency in subjects is reportedly cardioprotective [60], and individuals possessing a functional polymorphism associated with approximately two-fold decrease in MPO expression have reduced cardiac risks [61,62]. Given the increasing volume of pre-clinical and clinical data about the association of this enzyme with oxidation, atherogenesis and possibly plaque rupture, clinical trials have been initiated to assess its validity as a diagnostic and prognostic marker of myocardial ischemia.

In a landmark study, Brennan et al [63] measured baseline levels of MPO, troponin T, and CRP in 604 chest-pain patients. To establish normal MPO levels, researchers also obtained measurements from 115 healthy volunteers without coronary artery disease. MPO levels were higher in patients who had an MI within 16 hours after presentation than in patients who did not. An elevated level predicted risk even in patients with normal initial troponin levels and irrespective of the time between symptom onset and presentation. The risk increased with increasing quartiles of MPO level. Most important, elevated levels at presentation predicted the risk for major coronary events at 30 days and at 6 months. The adjusted odds ratio for patients in the highest quartile compared with patients in the lowest quartile was 4.7. MPO was found to be a stronger predictor than CRP. Last year, based mainly on the results of this study, FDA approved an enzyme-linked immunosorbent assay (CardioMPO) for the quantitative determination of MPO in human plasma. However, the general feeling of the medical community is that further studies are needed to gain more insight into the quantity and quality of information offered by this very promising new biomarker.

CONCLUSIONS

Acute coronary syndrome (ACS) is the final step of a complex pathophysiologic process including 1) progressive mechanical obstruction due to atheroma formation, 2) dynamic obstruction due to vasoconstriction, 3) plaque rupture with acute thrombosis as a consequence of oxidative stress and inflammation, 4) cardiac myocyte ischemia and necrosis, and 5) hemodynamic and ventricular wall stress. The better understanding of this whole process has led to the emergence of novel, sensitive biomarkers representing all the above pathophysiologic steps (Figure 1). There is growing evidence that these markers may become valuable for the diagnosis of ischemia and for assessing the risk of short and long term adverse outcome in patients presenting to the emergency department and to the outpatient clinic with symptoms suggestive of an ACS. However, these patients may vary substantially with respect to the relative contribution of each pathophysiologic step in the individual clinical picture. Accordingly, the relative importance of each biomarker may also vary in different patients. Moreover, both old and new biomarkers, although sensitive, lack the specificity needed to gain widespread clinical application as a single marker of disease. Thus, a multi-marker strategy employing a pathophysiologically diverse set of biomarkers seems the most appropriate to lead ultimately to the best therapeutic strategy for the individual patient [64]. Nevertheless, further work needs to be done to define the optimal weighing of each biomarker for diagnosis and prognosis of myocardial ischemia and to assess the appropriate therapeutic responses to different patterns of biomarker elevation in ACS.

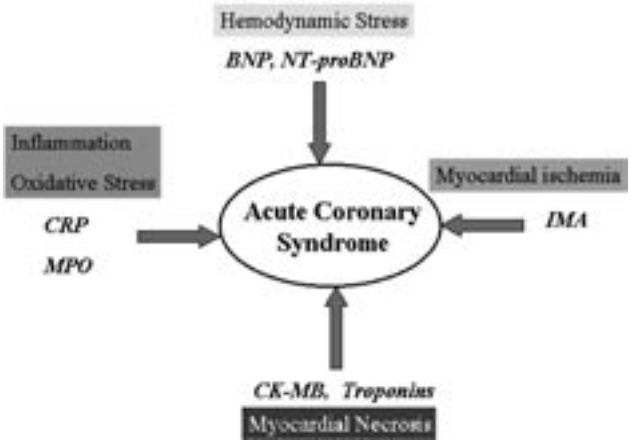

FIGURE 1. Association between biomarkes and pathophysiology in Acute Coronary Syndromes. BNP: Brain Natriuretic Peptide, NT-proBNP: N-terminal fragment – proBrain Natriuretic Peptide CRP: C-Reactive Protein, IMA: Ischemia Modified Albumin, MPO: Myeloperoxidase.

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